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Contents

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Current Affairs

Pulses – Changing scenario of diseases and their management strategies

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With more than 37 years of experience in research and development of pulses, Dr Om Gupta was associated with the development and release of ten high yielding, wilt resistant, early to medium varieties of chickpea for use in the state and CZ and also exploring of new techniques for the management of soil borne pathogens in pulses. She was also associated with reporting of three new diseases of chickpea for the first time from Madhya Pradesh and the identification of broad based multiple disease resistant genotypes on chickpea (wilt and dry root rot), being used as National donor parent for breeding disease resistant varieties. She has served as Principal Investigator (Crop Protection), AICRP on Chickpea at National level by ICAR (2006-2014), As first lady Dean in 2014 and in 2018 as first lady Director Extension Services in the history of Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur. She is member of the Chickpea team of JNKVV that is recipient of CGIAR'S 'King Baudonin Award 2002"and ICRISAT'S Doreen Mashler Award 2002, "Best Centre Performance Award 2006" (ICAR) and Millennium ICRISAT Science Award 2008 in chickpea research. She has >85 research papers to her credit apart from technical bulletins, manuals, book chapters and several review papers. She recently superannuated as Director Extension Services from JNKVV.

Pulses play a pivotal role in nutritional security and their soil ameliorative properties have been an integral part of sustainable agriculture since time immemorial. Globally, India ranks at top in terms of area under pulse crops with about 25 million tonnes of production. However, productivity of these protein dense crops is quite low which is associated with risk of crop failure due to several biotic, abiotic stresses and poor crop management practices.

Needless to mention that soil hosts a huge diversity of microbes, number of which vary with season being more in spring and fewer in winter and summer. Top layers of soil have more microorganisms than the deeper strata and are abundant in rhizosphere. Changing climate patterns are affecting the equilibrium of host pathogen interactions resulting in either increased epidemic out breaks or emergence of new pathogens or less known pathogens causing severe yield losses. Plant pathogens are among the first organisms to experience climate change evident via its population dynamics i.e. fecundity/ multiplication, virulence, survival and dispersal. Global climate change is being held responsible for the emergence of new diseases or for the transition of existing minor ones into major ones posing a serious threat to nutritional and food security.

Chickpea, pigeonpea, urdbean, mungbean, lentil and pea etc. are major pulse crops grown in India contributing to more than 90% to the total pulses production. These crops are largely grown in rainfed conditions, hence are most vulnerable to climate change which is often realized as intermittent droughts, extreme temperatures (heat, cold and frost), unseasonal rains, reduced number of rainy days etc.

Changes in diseases spectrum of chickpea and pigeonpea monitored through extensive surveys and analysis of long term diseases and weather data sets indicates emergence of new diseases and shifts in occurrence and distribution of diseases of these crops. In chickpea, frequent out breaks of diseases e.g. dry root rot (*Rhizoctonia bataticola*) is increasing due to higher temperature and terminal drought at pod formation stage particularly in rainfed conditions. The disease is more severe in legume vs legume cropping system in central and southern part of India. Similarly, collar rot (*Sclerotium rolfsii*) disease has become a major threat for chickpea cultivation under irrigated

condition, particularly in double cropping systems e.g. upland paddy followed by chickpea, lentil and pea. Chickpea rust (*Uromyces cicer arietini*) is an upcoming disease in eastern India and parts of Karnataka that has caused crop failure when there are few showers. In pigeonpea, *Phytophthora* blight (*Phytophthora cajani*) up to 25 days in early sown crop in Haryana, Punjab, Delhi, Uttarakhand, parts of Uttar Pradesh and Rajasthan and *Alternaria* blight (*Alternaria alternata*) in post rainy season crop at eastern Uttar Pradesh, Bihar, Jharkhand etc. are emerging faster with increased temperature and more frequent moisture stress. *Rhizoctonia* blight is now more intense in typically tropical humid areas while viruses and rust dominate in warm but dry zones. Data collected in India during the preceding years showed higher incidence of dry root rot in chickpea varieties those are resistant to *Fusarium* wilt in years when temperature exceeded 32°C at the time of flowering/podding. This is consistent with the observations recorded through green house experiments under manipulated soil moisture and temperature regimes revealing that *R. bataticola* infected chickpea plants caused dry root rot faster at 35°C temperature coupled with low soil moisture or equal to 60 percent. In contrast, cooler temperatures and wet conditions are associated with increased incidence of *Alternaria* blights in chickpea, lentil, pigeonpea, pea and lentil whereas anthracnose (*Colletotrichum* spp.) was observed in mungbean and urdbean. Recent studies indicate increased incidence and frequent out breaks of *Phytophthora* blight of pigeonpea in India over the last decade that can be attributed due to high intermittent rainfall during crop season.

Mungbean yellow mosaic virus (MYMV) in mungbean and urdbean are now becoming more prevalent disease as whiteflies (vector) transmit the disease from susceptible to healthy plants have developed new biotypes due to continuous use of same pesticide to manage its further transmission.

A number of management technologies to minimize losses due to diseases have been developed; however, huge crop losses are still often seen. Chickpea has shifted from highly productive irrigated condition of north India to rainfed areas in central and southern India. This has made the diseases viz., *Ascochyta* blight and *Botrytis* grey mould less frequent with wilt and root rots becoming important in newer nitches. Integrated management of these diseases needs specific focus on agro climate conditions, cropping pattern of different states with respect to use of resistant varieties and healthy seeds, modification of cultural practices, judicious use of fungicides and biocontrol agents.

Future prospects: Management strategies

Location specific integrated disease management modules developed for different diseases need to be refined and demonstrated and scaled up at farmers' field. These modules should be ecologically sound, economically viable and socially acceptable. Exploitation of cultural practices (deep plaughing in summer, crop rotation, intercropping, sowing time, sowing method, seed rate, plant spacing etc.) and use of fungicides coupled with resistant varieties hold great scope in the light of crop management.

Role of plant growth promoting rhizobia (PGPR) in improving the plant health/growth needs to be given more attention so that plants can check the entry of various pathogens. Development of efficient strains of bio-pesticides through biotechnological interventions is much needed to save the environment. Existence of high pathogenic variability in the newly developed varieties warrants for integration of various modern tools and techniques those are in hands of plant pathologists and plant breeders in pulses improvement programme. Understanding of pathogenic variability across the locations will aid to streamline the resistance breeding programme.

Development of multiracial resistant varieties needs to be focused upon. Screening of large number of drought or heat tolerant genotypes at hot spot location is to be executed to identify the sources of dry root rot resistance for their exploitation in chickpea breeding program. High level of target group participation to facilitate the area wide dissemination strategy for location specific integrated diseases managements (IDM) modules for pulse crops needs much more attention to improve economic, social and health status of the farming community.

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Review

Potential of biological control agents for the management of soil-borne pathogens in pulse crops

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ABSTRACT

Biocontrol of soil-borne pathogens of pulses is an emerging area of research and attracting researchers all over the world. Here, an attempt has been made to review the published research works on the biological control of soil-borne pathogen of pulse crops to analyze how progress has been made and what has been desired. Pulses, an important group of food crops are highly susceptible to soil-borne pathogens in India. Biocontrol, an eco-friendly approach, involves the use of beneficial microorganisms, their genes, and/ or products, such as metabolites, to control plant pathogens. The various mechanisms such as mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites and induction of plant defence system are involved in effecting typical biocontrol of a pathogen. Successful application of biocontrol strategies requires knowledge on the mode of action of the biocontrol agent to achieve better and effective management of a disease. To have more effective biocontrol strategies in future, it is crucial to carry out more research studies on some less understood aspects of biocontrol including the development of novel formulations, understanding the impact of environmental factors on biocontrol agents and the use of biotechnology and nanotechnology in the improvement of biocontrol mechanism and strategies. If implemented properly, the outlook of biocontrol of plant disease is bright and promising. The present article focuses on the history, screening, modes of actions, delivery systems of biocontrol agents and enhancement of biocontrol potential and application under field conditions to manage important soil-borne diseases of pulse crops.

***Key words*:** Biocontrol, Soil-borne pathogen, Pulse crops

INTRODUCTION

Pulses comprise many leguminous crops grown and consumed for their protein-rich grains. Pulses are particularly important in Indian society as the majority practices vegetarianism and rely mostly on pulse protein. These are an important group of food crops and are part of any healthy and balanced diet in India. These are reported to help prevent diseases such as cancer, diabetes, and heart disorders (Jukanti *et al*. 2012). Globally, pulses are grown in more than 171 countries. India is having the largest shares about 25 per cent of global pulses production from approximately 33 per cent of total world acreage under these crops. For the last five years, pulse production in India seems to have stabilized around 23-25mt. A look at the five years data indicates that the pulses productivity is around 0.8 t/h which is lower than that of the potential yields reported in the research stations. Biocontrol of soil-borne diseases of pulses can contribute to increasing the productivity of the pulses.

Pulses are generally grown under rain-fed resource-poor conditions. The improper sowing time, low seed rate, defective sowing method, lack of irrigation and low application of fertilizers are the major constraints to the productivity growth (Reddy 2009). Pulses are extremely vulnerable to several insect pests and diseases (Vijay *et al*. 2015). Among the diseases, soil-borne diseases have a severe impact on most of the economically important pulses in India (Table 1). In agriculture, pesticides may be employed to prevent, minimize, control, or manage soil-borne diseases of plants. However, these pesticides and chemicals are hazardous to the environment. Besides, they have been becoming more expensive and some are losing their efficiency owing to the development of resistant strains of pathogens. Therefore, it is imperative to look for eco-friendly approaches to control soil-borne plant diseases, which in contrast, should be cost-effective. The various microbes are commonly used as biofertilizers such as Bacteria, fungi, NPV, etc. The main reason which makes fungi

Table 1. List of important soil and seed-borne diseases of pulses in India

Major Pulses	Pathogens	Diseases	Nature of Pathogen
Chickpea	*Fusarium oxysporum* f. sp. *cicero*	Wilt	Seed and soil-borne
	Verticillium albo – atrum	Wilt	Soil-borne
	Pythium ultimum	Root and seed rot	Soil-borne
	Phytophthora megasperma	Root rot	Soil-borne
	Sclerotinia sclerotiarum	White rot	Soil-borne
	Rhizoctonia bataticola	Wilt	Soil-borne
Pigeonpea	*Macrophomina phaseolina*	Root rot	Seed and soil-borne
	Ascochyta rabiei	Blight	Seed and soil-borne
	Phytophthora dreschsleri	Blight	Soil-borne
	Fusarium udum	Wilt	Seed and soil-borne
Pea	*Fusarium oxysporum* f. sp. *pisi*	Wilt	Seed and soil-borne
	Ascochyta pisi	Blight	Seed and soil-borne
	A. pinodes	Blight	Seed and soil-borne
	A. pinodella	Wilt	Seed and soil-borne
	Sclerotinia sclerotiarum	White rot	Soil-borne
	Aphanomyces etueiches	Root rot	Soil-borne
Lentil	*Fusarium oxysporum* f. sp. *lentis*	Wilt	Seed and soil-borne
	Sclerotinia sclerotiarum	White rot	Seed and soil-borne
Mungbean	*Fusarium solani*	Root rot	Seed and soil-borne
Urdbean	*Rhizoctonia bataticola*	Root rot	Seed and soil-borne
	R. solani	Web blight	Soil-borne
	Sclerotinia rolfsii	Collar rot	Soil-borne
Rajmash	*Sclerotinia sclerotiarum*	White rot	Soil-borne
Lablab bean	*Fusarium oxysporum* f. sp. *phaseoli*	Wilt	Soil-borne

Source: Singh *et al.* (2016)

attractive biocontrol agents are ubiquitous, high specificity, host destruction, ease of culture maintenance in the laboratory. There are various genera such as *Trichoderma, Bauveria, and Glomus* etc which are effectively exploiting as biocontrol agents. Among the entire fungal species, *Trichoderma* spp.is a very popular and widely adopted biocontrol agent against several phytopathogens of pulse crops (Chaudhary and Prajapati 2004; Mishra *et al*. 2018a; Mishra *et al*. 2020a & b). Several species of Trichoderma were identified from pulses rhizosphere based on morphological characters. Morphological bases are insufficient for genus identification so, molecular identification was done using ITS and TEF markers (Mishra *et al.* 2016; 2020a). Undoubtedly, the use of biocontrol agents is an important and encouraging approach towards sustainable pulse production. This review presents recent progress in our understanding of the biocontrol of soil-borne pathogens, their status and future development and outlooks.

BIOCONTROL AGENTS (BCAS) RESEARCH AND DEVELOPMENT

During the past two decades, an urgent need was realized for management strategies that are safe for the environment and agriculture. Farmers are shifting towards eco-friendly technology for the management of diseases through BCAs or BCAs based formulations, referred to as bio-pesticides. The most important BCAs are *Trichoderma* spp., *Pseudomonas* spp., *Bacillus* spp., *Agrobacterium radiobacter*, nonpathogenic *Fusarium* spp., *Coniothyrium.* spp and *Aspergillus niger, Bacillus thuringiensis* (Bt), *Metarhizium* spp., *Beauveria bassiana* and nuclear polyhedrosis virus (NPVs), which are popularly used in plant protection. According to a recent report (NAAS 2013), nearly 1400 BCAs products were sold and 175 biopesticide active ingredients and 700 products were registered worldwide for their commercialization. A growing body of research articles report on the identification and efficacy of different BCAs against several pests and pathogens, however, their slow embrace is evident from the fact that only 2% of bio-pesticides are currently used for crop protection worldwide. Various carrier-based formulations available worldwide, alginate pellet- and talc-based formulations of BCAs have emerged as the most important carrier for the application in the management of crop diseases. This, however, has yielded inconsistent performance given that a single agent might not remain active in all soil conditions. Further, enabling mass production with a high level of microbial count and viability also assumes greater significance.

MECHANISMS OF BIOCONTROL

Biocontrol is defined as the inhibition of growth, infection and reproduction of an organism by another organism (Cook 1993; Baker 1987). It makes use of natural enemies of pests or pathogens to eradicate or control their population. Understanding the biology of interaction of biocontrol agents and pathogens may allow us to manipulate the soil environment to create conditions conducive for successful biocontrol or to improve biocontrol strategies (Chet 1987; Rovira 1965). The species of *Trichoderma* Fr and *Gliocaldium* Corda, *Pseudomonas fluorescens, Bacillus subtilis* and *B. brevis* have been identified as potential biocontrol agents against the soil-borne pathogen in pulses (Papvizas 1985; Chet 1987; Mukhopdhyay 1987; Mukhopdhyay *et al*. 1992; Mukherjee and Tripathi 2000). Other rhizospheric microorganisms like *Verticillium chlamydosporum* (Siddiqui and Mahmood 1996), *Gliocaldiumroseum* (Xue 2000), *Coniothyrium minitans* (Cael *et al*. 2001) provide biocontrol through a mechanism such as the production of antibiotics (Bender *et al*. 1999), iron sequestering compounds, siderophores (Dwivedi and Johri 2003; Siddiqui 2006), extracellular hydrolytic enzymes (Fridlender *et al*. 1993), other secondary metabolites such as hydrogen cyanide (HCN) (Pal *el al.* 2000; Validov *et al*. 2005) and induced systemic resistance (Ongena *et al*. 2004). Biological control is a result of many different types of interaction among microorganisms. The type of antagonism is (i) fungi stasis (ii) antibiosis (iii) lysis (iv) inhibition by competition and (v) mycoparasitism and predation (Audenaert *et al*. 2002; De Meyer and Hofte 1997; Elad and Baker 1985; Homma *et al*. 1989; Islam *et al*. 2005; Mezine *et al*. 2005; Ryu *et al*. 2004; Van Dijk and Nelson 2000). Different interactions taking place in the process of biocontrol are briefly described here.

Mycoparasitism

Mycoparasitism refers to the parasitism of one fungus by another. Several hyperparasites of soil-borne plant pathogens have been exploited in biocontrol (Table 2). The mechanism of hyper parasitism includes different kinds of interaction like coiling of hyphae

Table 2. Fungi as biocontrol agents of plant disease

Crop	Disease	Pathogen	Bio-agent	Effects	References
Mung	Dry root-rot	*Macrophomina phaseolina*	*Trichoderma harzianum*	Best survival of plants	Mani and Marimuthu, 1994
Mash bean	Root-rot	*M. phaseolina*	*T. hamatum, Gliocladium virens*	No infection occurred	Shahzad *et al.* (1991)
Pea	Damping-off	*Pythium* sp.	*T. hamatum, T. koningii*	Pre-emergence damping-off reduced	Lifshitz *et al*. (1986)
Pea	Seedling disease	*Rhizoctonia solani*	*Penicillium vermiculatum*	Seedling disease prevented	Boosalis (1956)
Soybean	Root disease	*R. solani, M. phaseolina, Fusarium* sp.	*Paeciliomyces lilacinus, G. virens, T. harzianum*	Root infection significantly reduced	Ali and Ghaffar (1991)
Chickpea	Wilt	*Fusarium oxysporum* f. sp. *ciceri*	*T. harzianum*	30% decrease in incidence, 50% decrease in severity	Khan *et al.* (2004)
Chickpea	Wilt	*Fusarium oxysporum* f. sp. *ciceri*	*T. harzianum*	8% decrease in disease incidence after 60 days and 11.1% decrease in disease incidence after 90 days	Prasad *et al*. (2002)
Chickpea	Chickpea wilt complex	*Fusarium oxysporum* f. sp. *ciceri*	*T. harzianum*	53.5 – 85.7% reduced incidence of chickpea wilt complex	Kaur and Mukhopadhyay (1992)
Chickpea	Wilt	*F. oxysporum* f. sp. *ciceri*	*T. harzianum*	87% reduction in wilt	Jayalaxmi *et al*. (2009)
Pigeonpea	Wilt	*F. udum*	*T. harzianum*	Lowers wilt incidence 20%	Jayalaxmi *et al*. (2003)
Pigeonpea	Wilt	*F. udum*	*T. harzianum*	89% reduction in disease	Biswas and Das (1999)
Chickpea	Wilt	*F. oxysporum* f. sp. *ciceri*	*T. harzianum*	78.44 % growth inhibition of pathogen	Mahajan *et al.* (2020)

around the pathogen, penetration, production of haustoria and lysis of hyphae (Chet *et al*. 1981; Goldman *et al*. 1994). Adams (1990) defined the efficiency of biocontrol agents as the ratio of the number of mycoparasites propagules required to obtain disease control to the typical inoculum density of a plant pathogen. He demonstrated that 5 x 10^6 CFUs of *Trichoderma* were required to control *R. solani*. Khan and Khan (2003) found that the application of *T. harzianum* decreased the incidence and severity of wilt by 42 % and 39 % in the case of *T. virens*. The efficacy of the biocontrol agent depends on the sickness level of the field soil (Ghante *et al.* 2019). Mahajan *et al.* (2020) reported 78.44 per cent growth inhibition of chickpea wilt disease pathogen (*Fusarium oxysporum* f. sp. *ciceri*)by local isolate *T. harzianum* (Th-III). Recent advances in molecular analysis, including the cloning of a *T. harzianum* gene encoding endochitinase and methods of transformation of *Trichoderma* and *Gliocladium* make the generation of mutants with multiple gene disruptions feasible (Hayes *et al*. 1994). *T. hamatum* have been used effectively in controlling *Pythium* species and *Rhizoctonia solani* responsible for causing damping off disease in the seedlings of several plants and *Corticium rolfsii* which causes root rot. The primary antagonistic relationship between *T. hamatum* and these pathogens involve hyphal coiling and penetration (Mukhopdhyay *et al*. 1986). *T. harzianum* exhibits excellent mycoparasitic activity against *Rhizoctonia solani* (Altamare *et al*. 1999), strain IE – 2 and IE – 6 of *Pseudomonas aeruginosa* lysed the fugal mycelium of *Macrophomina phaseolina, Fusarium solani* and *Rhizoctonia solani* used as a seed dressing or a soil drench significantly suppressed root-rot – root-knot disease complex of mungbean (Ali *et al*. 2001). Delivering *T. harzianum* through the soil, during sowing increased the percentage of survival of peanut (90%), while in control none of the plants survived (Muthamilan and Jeyarajan 1996).

Coniothyrium minitans is a potential mycoparasite against *Sclerotinia* spp. Application of *C. minitans* inoculum to soil has been reported to reduce the survival of sclerotia of *Sclerotinia sclerotiorum* (Cael *et al*. 2001; Turner and Tribe 1975). The antagonistic potential of *Trichoderma* isolates was assessed through the binary culture technique. The inhibition percentage of pathogen mycelia of all the pathogenic fungi (*Fusarium udum, Fusarium oxysporum lentis* and *Fusarium oxysporum ciceri)* was recorded for all the strains (Mishra *et al*. 2018b). A Nobel bacteria identified as *Bacillus altitudinis* (MT641195) has also been identified which have antagonistic action against *M. phaseolina* and *P. drechsleri* f. sp. *cajani* (Mishra *et al*. 2020c).

Antibiosis

The antagonistic organism releases antibiotics or other chemicals which are harmful to the pathogens and inhibit their growth (Homma *et al*. 1989; Howell and Stipanovic 1980; Islam *et al*. 2005; Shanahan *et al*. 1992; Thomashow and Weller 1988) (Table 3). A few highly effective disease–suppressive agents are found among the fluorescent Pseudomonads, making this group of bacteria the most widely studied group of antibiotic producers in the rhizosphere. *Pseudomonas fluorescens* strain CHA0 produces hydrogen cyanide, 2, 4 – diacetyl phloroglucinol and pyoluteorin, which directly interfere with the growth of various pathogens and contribute to disease suppression (Keel *et al*. 1992; Maurhofer *et al*. 1994; Voisard *et al*. 1989) (Table 4, Fig 1). An emerging theme in the fluorescent pseudomonads is that global regulatory elements coordinate the production of secondary metabolites. For instance, biosynthesis of phenazine derivatives in *P. aureofaciens* is involved in the biocontrol of *F. oxysporum* on diverse crops (Chin –A Woeng *et al*. 2001a, 2001b and Sylvie *et al*. 2009). Also, the antibiotic 2, 4 – diacetyl phloroglucinol (DAPG) has substantial

Table 3: Bacteria as bio-control agents of plant disease

Crop	Disease	Pathogen	Bio-agent	Effects	References
Pigeonpea	Wilt	*Fusarium udum*	*Bacillus subtilis*	31% seedling dry weight stimulated.	Podile and Dube (1988)
Pigeonpea	Wilt	*F. udum*	*B. subtilis*	13% increase in seedling length and 23% increase in seedling dry weight.	Podile (1995)
Soybean	Root disease	*Macrophomina phaseolina, Rhizoctonia solani, Fusarium* sp.	*B. subtilis, Streptomyces* sp.	The pathogen was significantly suppressed.	Ali and Ghaffar (1991)
Soybean	Root disease	*Sclerotium rolfsii, Rhizoctonia bataticola, Fusarium* sp.	*B. subtilis*	Pathogen association with soybean decreased.	Kumar and Khare (1990)
Pigeonpea	Wilt	*F. udum*	*B. subtilis*	88% reduction in wilt	Vasudeva *et al.* (1963)

Table 4. Antibiotics or antibiotic like effectors produced *Bacillus subtilis* and *Pseudomonas fluorescens*

Antibiotic	Organism	Reference
Bulbiformin	*B. subtilis*	Vasudeva *et al.* (1952); Brannen (1995)
Iturin B	*B. subtilis*	Asaka and Shoda (1996)
Iturin A	*B. subtilis*	Kloepper *et al.* (2004)
Surfactin	*B. subtilis*	Edwards and Seddon (1992)
Agrocin-84	*B. subtilis*	Kim *et al.* (1997)
Bacillomycin	*B. subtilis*	Besson and Michel (1984)
Mycosubtilin	*B. subtilis*	Leclere *et al.* (2005)
Fengymycin	*B. subtilis*	Vanillakam and Lowffler (1986)
Mycobacillin	*B. subtilis*	Sengupta *et al.* (1971)
Bacillomycin D	*B. subtilis*	Moyne *et al.* (2001)
Pyoluteorin	*P. fluorescens*	Whistler *et al.* (2000)
Phenazin	*P. fluorescens*	Schoonbeck *et al.* (2002)
Indole acetic acid	*P. fluorescens*	Mordukhova (2000)
N-butylbenzenesulphonamid	*P. fluorescens*	Kim-Keunki *et al.* (2000)
Siderophores	*P. fluorescens*	Perez *et al.* (2001)
Oomycin A	*P. fluorescens*	Gutterson *et al.* (1988)
Alginate, HCN, Pseudomonic acid Oomycin, 2-Hydroxy-2,4,6-cyclo Hepta-triene-1-pseudomonic acid, Ovafluorin, Fluopsin C&F, SorbistinA1&B, Salicylic acid	*P. fluorescens*	Johri *et al.* (1997)

activity against pathogenic *F. oxysporum* (Schourten *et al*. 2004).

Although bacilli have received less attention as potential biocontrol agents than the pseudomonads, evidence indicates that they may promote effective disease suppression. They produce stable endospores, which can survive the heat and desiccation conditions that may be faced by biocontrol agents (Turner and Backman 1991; Lumsden *et al*. 1995; Osburn *et al*. 1995). Analysis of mutants of *B. cereus* shows a significant quantitative relationship between disease suppressiveness and the production of two antibiotics, zwittermicin A and kanosamine (Silo-Suh *et al*. 1994; Milner *et al*. 1996). The purified antibiotics suppress disease and inhibit the development of oomycetes by stunting and deforming germ tubes of germinating cysts.

Bacillus subtilis releases some antibiotics in rhizosphere and helps lower wilt in pigeonpea (Vasudev and Roy 1950; Vasudeva *et al*. 1958)). Seed bacterization with *B. subtilis* AF 1 enhanced growth parameters of pigeonpea (Podile and Dube 1988; Podile (1995) and Manjula and Podile (2001) reported that chitin supplemented formulation of *B. subtilis* improved pigeonpea plant dry weight by 2-28%. A reduction by 88 % in the incidence of pigeonpea wilt was noticed in the autoclaved soil containing molasses, sweet clover roots and groundnut cake and inoculated with *B. subtilis* (Vasudeva *et al*. 1963). It was explained that a higher amount of bulbiformin produced by the bacteria became systemic in the plant and provided a protective zone around the roots of pigeonpea seedlings (Singh *et al*. 1965).

Saikia *et al.* (2003) observed a 33% increase in root length and a 10% increase in shoot length in chickpea plants that received soil treatment of *P. fluorescens* isolate Pf 4-29. *Trichoderma* and *Gliocladium* are closely related funga1 biocontrol agents. Each produces antimicrobial compounds and suppresses disease by diverse mechanisms, including the production of the structurally complex antibiotics gliovirin and gliotoxin (Howell *et al*. 1993) (Table 5). Mutants of *Gliocladium virens* that do not produce gliotoxin are reduced in their ability to control *Pythium* damping-off (Wilhlte *et al*. 1994). Mutants with increased or decreased antibiotic production show a corresponding effect on biocontrol (Howell and Stipanovic 1983).

Iron Competition

Biocontrol agents suppress the pathogen by depriving it of nutrients (Elad and Baker 1985; Keel *et al*. 1989; Loper and Buyer 1991). Iron competition is important in biological disease control. In highly oxidized and aerated soil, iron is present in ferric form (Kageyam and Nelson 2003; Shahraki *et al*. 2009) which is insoluble in water and the concentration may be extremely low. Organisms were found to secrete iron-binding ligands called siderophores having a high ability to obtain iron from the microorganisms to

Table 5. Antibiotics or antibiotics-like effectors produced by *Trichoderma* species.

Antibiotic	Reference
Trichodermin	Gotfredson and Vangedal (1965)
Dermadin	Pyke and Dietz (1966)
Trichoviridin	Yamano *et al.* (1970)
Sequiterpeneheptalic acid	Itoh *et al.* (1980)
Trichorzianines, Trichorviridin, Propionic acid, 3-(3-isocyanocyclopent-2-enzylidene), Acrylic acid, 3-(3-isocyano-6-oxabicyclo (3, 10) hex-2-eh-5-yl	Baldwin *et al.* (1981)
Chitinase	Elad *et al.* (1982)
6-n-pentenyl-2H-pyran-2 one, 6-n-pentenyl-2H-pyran-2-one	Claydon *et al.* (1987)
Alamethicine, Paracelsin, Trichotoxin	Lumsden *et al.* (1991)
Harzianolide [3-(2-hydroxyl-propyl) -4(hexa-2"-dienyl-2(5H) furanone	Claydon *et al.* (1991)
Chitin-1-4-β-chitobiosidase n-acetyl, β-D glucosaminase, Endochitinase	Harman *et al.* (1993)
Heptelidic acid	Howell *et al.* (1993)
Chitobiase	Ulhaa and Peberdy (1993)
Protease	Elad *et al.* (2000)
β-1,3-glucanase	Perez *et al.* (2001)
α-gluxosidase protein	Shanmugam *et al.* (2001)
Gliotoxin, Trichodermin, Viridin	Haggag and Mohamed (2002)
Trichosetin	Marfori *et al.* (2002)

survive in such an environment (Shahraki *et al*. 2009). The fluorescent pseudomonads produce a group of siderophores known as the pseudobactins, pyoveridine, colourlessnacrdamine, pyochelin, salicylic acid and cephactin which are structurally complex iron-binding molecules (Singh *et al*. 2016). Analyses of mutants lacking the ability to produce siderophores suggest that they contribute to the suppression of certain fungal and oomycete diseases (Duijff*et al*. 1994; Buysens *et al*. 1996). Elad and Baker (1985) observed a direct correlation between siderophore synthesis in fluorescent pseudomonads and their capacity to inhibit germination of chlamydospores of *F. oxysporum*. They found that chlamydospore germination at 0.0 optical density was 80 %. The increased concentration of siderophore of pseudomonads (0.15 optical density) reduce the germination to 12.6 % (r = 0.747).

Bacterization of chickpea seeds with a siderophore-producing fluorescent pseudomonad RBT 13 reduced the number of chickpea wilted plants in wilt sick soil by 52 per cent (Kumar and Dube, 1992). Vidyasekaran *et al.* (1997) reported that out of 27 fluorescent pseudomonad strains, five were significantly effective against *F. udum in vitro* and all of them were identified as *P. fluorescens*. Of the five isolates, two strains, Pf1 and Pf2 consistently showed high levels of inhibitory activity producing inhibition zones of 69 and 62 mm respectively. In pea, seed treatment with *P. fluorescens* isolates 63-28 brought about the formation of structural barriers such as cell wall apposition (papillae) and deposition of newly formed callose and accumulation of invading hyphae of *Pythium ultimum* and *Fusarium oxysporum* f.sp. *pisi* (Benhamou *et al*. 1996). According to Gaind and Gaur (1991), *Bacillus subtilis* inoculants increased biomass grain yield and P and N uptake of mung bean. Further work is needed to characterize the ability of soil-borne organisms to utilize siderophores produced by biocontrol agents. Rapid breakdown of biocontrol would be expected if the target pathogens could circumvent disease suppression predicated on iron deprivation by acquiring the ability to utilize the siderophores from their neighbours in the soil.

Induction of resistance

Certain biocontrol agents not only affect the pathogen but also induce resistance in the host plant often referred to as induced plant resistance (Audenaert *et al*. 2002; Vallad and Godman 2004). This induced resistance is of two types representing two distinct pathway responses: systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Table 6). Typically, SAR is mediated by salicylic acid, a chemical compound produced after pathogen infection that leads to the expression of pathogenesis-related (PR) proteins such as PR-1, PR-2, chitinases, and some peroxidases (Kageyama and Nelson 2003; Ramamoorthy *et al*. 2001; Vallad and Godman 2004). These PR proteins can cause lysis of invading cells, reinforcement of cell membranes to resist infections or induce localized cell death (Vallad and Godman 2004). *Trichoderma* species produce a 22 k Da xylanase that, when injected in plant tissues, will induce plant defence responses including K^+, H^+ and Ca^+ chanelling, PR protein synthesis, ethylene biosynthesis and glycosylation and fatty acylation of phytosterols (Bailey and Lumsden 1998). Pectic

Table 6. Bacterial determinants and types of host resistance induced by *Bacillus subtilis* and *Pseudomonas fluorescens.*

Biocontrol - Bacteria	Strain	Plant Species	Bacterial determinant	Type	Reference
Bacillus subtilis	GB03	*Arabidopsis*	2,3-butanediol	ISR	Ryu *et al.* (2004)
B. subtilis	IN 937	*Arabidopsis*	2,3-butanediol	ISR	Ryu *et al.* (2004)
B. subtilis	CHAO	Tobacco	Siderophore	SAR	Mourhofer *et al.* (1994)
Pseudomonas fluorescens	CHAO	*Arabidopsis*	Antibiotics	ISR	Iavicoli *et al.* (2003)

oligogalacturonides released after hydrolysis by a non-pathogenic binucleate *Rhizoctonia* isolate may act as elicitors of defence responses in French beans (Jabaji *et al.* 1999). Seed bacterized with *B. subtilis* significantly reduced the incidence of pigeonpea wilt and an increase in phenylalanine ammonia lyase (PAL) and peroxidase activities in the host plant (Podile and Laxmi 1998; Harish *et al*. 1998).

A second pathway called ISR, is salicylic acid independent and is mediated by jasmonic acid and/or ethylene, which are produced by non-pathogenic rhizobacteria (Audenaert *et al*. 2002; De Meyer and Hofte 1997; Klopper *et al*. 1980; Leeman *et al*. 1995c; Moyne *et al*. 2001; Van Loon *et al*. 1998; Van Peer and Schippeers 1992; Van Wees *et al*. 1997). ISR was first observed on carnation with reduced susceptibility to wilt caused by *Fusarium* sp. (Van Peer *et al*. 1991). ISR results in strengthening of plant cell wall and alteration of host plant physiology and metabolic responses, leading to an enhanced synthesis of plant defence chemicals upon challenge by pathogens and/or abiotic stress factors (Ramamoorthy *et al*. 2001). Some strains of root colonizing microorganisms have been identified as potential elicitors of plant host defences. For instance, some biocontrol active strains of *Pseudomonas* species and *Trichoderma* species are recognized to induce plant host defences (Haas and Defago 2005; Harman *et al*. 2004). Paromarto and coworkers (1988) implied that induce resistance is the mechanism of biocontrol of *Rhizoctonia solani* on soybean by binucleated *Rhizoctonia solani*.

Two antagonistic fungi *Trichoderma harzianum* and *T. viride* were found to be effective against the natural incidence of wilt and wet root rot of the chickpea when applied to soil one week before sowing and then seed treatment in reducing the disease. The wilt incidence was higher (12 and 16%) in the control plot, but in *T. harzianum* soil treated plants only 4 and 5.1% wilt incidence were observed at 60 and 90 days after sowing respectively (Prasad *et al*. 2002). Soil application of *T. harzianum* reduced 53.5-85.7 per cent incidence of chickpea wilt complex (Kaur and Mukhopadhyay 1992), whereas seed treatment with *T. harzianum* decreased wilt incidence and severity in chickpea (Khan *et al*. 2004) and pigeonpea (Jayalakshmi *et al.* 2003). Dubey and Singh (2009) reported that *Trichoderma* species were superior to *B. subtilis* and *A. niger* to control wilt of chickpea by *F. oxysporum* f. sp. *ciceri.* Jaylakshmi *et al* (2009) reported up to 87% reduction in chickpea wilt caused by *F. oxysporum* f.sp. *ciceri* after seed treated with *T. harzianum* sown in wilt sick plot containing *T. harzianum* L1 biomass with crab shell powder. Coating chickpea seeds with biocontrol agent *B. subtilis, G. virens, T. harzianum* and *T. viride* reduced wilt (De *et al.* 1996). Seeds coated with *T. viride* increased the fresh and dry weight of shoot, root, and nodules of broad beans (Woo *et al*. 2006). In another study by Kumar *et al* (2007) found that *P. fluorescens* inhibited the mycelium growth of *M. phaseolina*, reduced the disease severity, and significantly increased the biomass of the chickpea plants. Saikia *et al.* (2003) reported that isolates of *P. fluorescens* systematically induced resistance against *Fusarium* wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* (*Foc* Race1), and significantly (P=0.05) reduced the wilt disease by 26-50% as compared to control.

Moreover, in suppression of *Fusarium* wilt by *P. fluorescens*, preparations of lipopolysaccharides from the bacterial cell surface induce resistance as effectively as the living bacteria, demonstrating that biocontrol is not necessarily due to transport of the bacteria or an antibiotic through the plant (Leeman *et al*. 1995a, 1995b). Whether or not biocontrol agents suppress disease by inducing resistance, SAR and biocontrol strategies must be compatible, because future agricultura1 practices are likely to require the integration of multiple pest control strategies (Chen *et al*. 1996).

Colonization on host plant

Root colonization ability of biocontrol agents and potential to survive and proliferate along with growing roots over a considerable period, in the presence of the indigenous microflora results in intimate associations that directly provide a selective adaptation to plants towards specific ecological niches (Lugtenberg and Dekkers 1999; Parke 1991; Whipps 1997). Also, the ability of biocontrol agents to colonize specific substrates or sites, whether a seed, root, shoot area, stump, or fruit surface (Parke 1991), protects the infection site from pathogen attack. In the suppression

of damping-off of peas by *P. cepacia* (renamed *Burkholderia cepacia*), there is a significant relationship between the population size of the biocontrol agent and the degree of disease suppression (Parke 1990). It has been proposed that the growth of both the AM fungi and root pathogens depends on host photosynthates and that they compete for the carbon compounds reaching the root (Sharma *et al*. 1992; Linderman 1994). Cordier *et al*. (1996) showed that *Phytophthora* development is reduced in AM fungal colonized and adjacent uncolonized regions of AM root systems and that in the former the pathogen does not penetrate arbuscular containing cells.

Plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas* and *Bacillus* strains are the major root colonizers (Manikanda *et al*. 2010; Joseph *et al*. 2007) and can elicit plant defences (Kloepper *et al*. 2004) (Table 7). Combined inoculation with *Glomus intraradices*, *Pseudomonas putida* and *Paenibacillus polymyxa* highly reduced galling, nematode multiplication, and root disease complex (*Meloidogyne incognita* and *Macrophomina phaseolina*) of chickpea, while root colonization by arbuscular mycorrhizal fungus, *G. intraradices* was increased in the presence of *P. putida* and *P. polymyxa* (Akhtar and Siddiqui 2007). Several species of *Glomus* are implicated in the reduction of root diseases viz., *G. intraradices* reduced in root – rot of pea caused by *Aphanomyces etueiches* (Bodkar *et al*. 1998). Zaidi and Khan (2006) reported that triple inoculation of *Glomus fasciculatum, Bradyrhizobium* sp. (Vigna) and *B. subtilis* increased dry matter, yield, chlorophyll content in foliage and N and P uptake of a green gram; plant seed yield was enhanced by 24%. Chand *et al.* (1991) found that VAM endophyte, *Glomus mossae* reduced the wilt incidence from 80% to 10 % in the VAM treated plants.

Metabolite production

Many biocontrol agents produce other metabolites that can interfere with pathogen growth and activities. Various extracellular hydrolytic enzymes produced by microbes play important role in the suppression of plant pathogens. Chitinase and ß-1, 3-glucanase attack on chitin and ß -1, 3-glucan, major constituents of many fungal cell walls (Anderson *et al*. 2004; Lam and Gaffney 1993; Wilhlte *et al*. 2001), resulting in its degradation which further kills the pathogens (Chernin and Chet 2002). Dukare and Paul (2021) evaluated disease suppression and plant growth promotion ability of *Pseudomonas* sp. NS 1 and *Bacillus* sp. NS 22 in pigeon pea wilt *in vivo* and found that the *F. udum* mycelia growth and biomass *in vitro* was inhibited and wilt disease severity was reduced of plants grown in Fusarium-infested soil. They found that fungicidal action of these rhizobacteria was due to the production of numerous biocidal compounds including different antifungal metabolites, chitinolytic (endochitinase, exochitinase, chitobiase) and other cell wall degrading lytic enzymes (proteinase, cellulose, amylase, pectinase, lipase), siderophores, and antifungal volatile compounds such as ammonia and cyanide.

Chitinase produced by *Serratia marcescens*, *S. plymuthica*, *Paenibacillus* sp. and *Streptomyces* sp. was found to be inhibitory against *Botrytis cinerea*, *Sclerotium rolfsii*, *Fusarium oxysporum* f. sp. *cucumerinum* (Ordentlich *et al.* 1988). Similarly, laminarinase produced by *Pseudomonas stutzeri* digest and lyse mycelia of *F. solani* (Lim *et al.* 1991). ß -1, 3-glucanase synthesized by *Paenibacillus, B. cepacia* destroy *F. oxysporum, R. solani, S. rolfsii, and Pythium ultimum* cell walls (Fridlender *et al.* 1993). It seems more likely that

Table 7: Plant growth-promoting micro-organism as bio-control agents of plant disease

Crop	Pathogen	Micro-organisms	Effects	References
Pea and soybean	*Macrophomina phaseolina*	*Rhizobium japonicum*	Significant suppression of the disease.	Chakraborty and Chakraborty (1988)
Bean	*Fusarium solani*	*Pseudomonas putida*	The pathogen population decreased.	Andeseon and Guerra (1987)
Mungbean	*M. phaseolina*	*VAM*	Significant reduction in the disease incidence.	Jalali *et al.* (1990)
Mungbean	*M. phaseolina*	*Glomus fasciculatum*	Yield increased.	Jayaraman (1991)
Chickpea	*Sclerotium rolfsii*	*G. fasciculatum*	Yield increased.	Jayaraman (1991)
Chickpea	*Fusarium oxysporum* f. sp. *ciceri*	*G. mosseae*	Wilt incidence reduced from 80% to 10%.	Chand *et al.* (1991)
Chickpea	*Fusarium oxysporum* f. sp. *ciceri*	*P. fluorescens*	33% increase in root length and 10% increase in shoot length	Saikia *et al*. (2003)
Pigeonpea	*F. udum*	*Pseudomonas* sp. NS 1 and *Bacillus* sp. NS 22	inhibited *F. udum* mycelia growth and biomass *in vitro* and reduced wilt disease severity in plants grown in controlled environmental conditions	Dukare and Paul (2021)

antagonistic activities of these metabolites are indicative of the need to degrade complex polymers to obtain carbon nutrition.

Genetic evidence for the role of these enzymes in biocontrol has been obtained where *ChiA* from *S. marcescens* was inserted into the non-biocontrol agent *Escherichia coli* and the resulting transgenic bacterium reduced disease incidence of Southern blight of bean caused by *Sclerotium rolfsii* (Shapira *et al*. 1989). Similarly, transformed *Trichoderma harzianum* with *ChiA* from *S. marcescens* (Haran *et al*. 1993) was more capable of suppressing *Sclerotium rolfsii* than the original strain. Recently, a trademark in the history of biocontrol was established by generating transgenic plants containing the gene for endochitinase from *T. harzianum* with increased resistance against plant pathogenic fungi (Lorito *et al*. 1993a). These results indicate that these enzymes play an important role in biocontrol and the biocontrol ability of some microbes may be improved by transformation with chitinolytic enzymes.

BIOPESTICIDES

Biopesticide formulations based on bacteria, fungi, viruses, nematodes, protozoa etc. are known as microbial pesticides. These microbial pesticides also include antagonistic organisms for the biological control of plant diseases. Microbes such as *Bacillus subtilis, Gliocladium* spp., *Trichoderma* spp., *Pseudomonas fluorescens, Beauvaria bassiana, Metarrhizium anisopliae, Verticillium lecanii,* granulo- and nuclear polyhedralviruses (NPV) have been added in a schedule vide by doing amendment in Insecticides Act, 1968 to be used for commercial production as a biopesticide. This has been published in the Gazette of India dated March 26, 1999 and many more have been included in this schedule for the production of microbial biopesticides.

The present scenario in India represents only 4.0 per cent contribution of biopesticides whereas the major part is still held by the insecticides and fungicides (Glare *et al*. 2012). Across the world, the increase in the share of biopesticides has been major in Europe with an average annual growth rate (AAGR) of 15 per cent followed by that in Asia with AAGR of 12 per cent and a minute increase of 5% was noticed in the Latin America (Industrial Equipment Newsletter). This development signifies the rising tendency in the developing countries for promoting biocontrol strategy of disease management as an initiative towards the most awaited evergreen revolution.

DELIVERY SYSTEM OF BIOCONTROL AGENTS IN PULSES

Biocontrol formulations are delivered through several means based on the survival nature and mode of infection of the pathogen. It is delivered through seed treatment, soil application, and foliar application or a combination of several methods.

Seed Treatment

Seed treatment is one of the most effective methods for the management of plant pathogens. In this treatment, hydration of seed is controlled to a level that permits pre-germinative metabolic activity to take place without the emergence of the radical. Treating pigeon pea and chickpea seeds with the talc-based formulation of *T. harzianum, Trichoderma viride, T. hamatum, T. virens, Bacillus subtilis,* and *Pseudomonas fluorescens* facilitates the management of Fusarium wilt in both crops (El-Hassan and Gowen 2006; Dubey *et al.* 2009). *In-vitro* and *in-vivo* demonstrations have evidenced that the seed treatment with a talc-based formulation of Dalhanderma (*T. asperellum*) led to a decrease in wilt and root rot incidence in chickpea, pigeonpea and lentil, respectively (Mishra *et al*. 2019, 2020b).

Soil Application

The fully active growing population of bioagents is applied in the soil at the time of sowing. The formulations of *Trichoderma* may be used for soil application as well as drenching at the initial growth stage of the crops. According to Vidhyasekaran and Muthamilan (1995), soil application of peat-based formulation with *P. fluorescens* (Pf1) at 2.5 kg of formulation mixed with 25 kg of well-decomposed farmyard manure improved management of chickpea wilt. Combining *P. fluorescens* with safer fungicides reduced the wilt complex in pigeon pea (Siddiqui 2006).

CONCLUSION AND FUTURE OUTLOOK

It is now widely recognized that the biocontrol of plant pathogens is a distinct possibility for the future and can be successfully exploited in modern agriculture, especially within the framework of the integrated pest management system. The success of biocontrol of plant diseases is dependent on the intricate array of interactions. There is a need to understand such interactions at the molecular and ecological levels so that the strategies can be developed to use biocontrol in agriculture. The integrated use of genetic, molecular, and ecological approaches will

form the basis for significant future advances in biocontrol research (Spadaro and Gullino 2005).

Few issues have been identified that need to be addressed for making practical use of biocontrol strategies for agriculture in India: Firstly, better strategies should be devised for the screening of biocontrol agents. Super strains with augmented biocontrol efficacy should be developed. Strategies to minimize the resistance of pathogen to biocontrol agents and prevent its spread should be designed. Secondly, the genetics of the host should be exploited for the supportiveness of biocontrol, and hospitality to biocontrol agents should be enhanced through directed breeding or genetic modification of the host plant. Thirdly, a better understanding of microbial community ecology is needed. Before commercializing the biopesticides, sufficient focus should be given to their adaptability to different types of soil and agroclimatic situations. Therefore, it is needed to develop a microbial consortium for different soil types and agroclimatic zones. Fourthly, molecular methods should be developed for the study of microorganisms in their environments and developed transgenics by exploiting and utilizing the useful genes from biocontrol agents. Fifthly, the farmers should be educated regarding the use of biopesticides and multidisciplinary approaches to integrate better biocontrol with IPM and other production issues to have a sustainable agricultural system with minimum risks to the environment. Sixthly, registration guidelines under the Insecticide Act 1968 should be relaxed particularly for toxicological data generation of those microbial species whose toxicological data are already available for different strains. Strict penalizing policies should be implemented for the producers of spurious biopesticide products. Further, plant protection scientists should aim at enriching soil (field) with antagonistic microbial communities to make soil suppressive to the soil-borne pathogens.

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Review

Persuasive solutions to bring back hybrid pigeonpea breeding programmes on the rails

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ABSTRACT

Pigeonpea is the only pulse crop where considerable natural cross-pollination occurs. To make use of this natural phenomenon, crop breeders first bred a cytoplasmic nuclear male sterility (CMS) system and then researched to develop a suitable hybrid breeding technology. This included the development of high-yielding single cross hybrids and their large-scale seed production system. These endeavors resulted in release of three pigeonpea hybrids that recorded 30-50% on-farm yield gains over the inbred controls. These hybrids, however, failed to reach farmers due to a single factor *i.e.* inability of seed producers to maintain high seed standards. In general, the hybrid seed quality in field crops is determined by applying the standard "Grow-out tests" (GoT). Unfortunately, in the three released hybrids, this approach could not be used due to their photo-period sensitivity and long generation turnover time. The advances in pigeonpea breeding and genomics research, however, have provided a couple of user-friendly solutions to overcome the limitations of seed quality maintenance. The authors conclude that investments in breeding of early maturing hybrids where GoT can be applied with ease and the use of genomics-based seed quality testing can bring the pigeonpea hybrid programs back on rails.

Key words**:** *Cajanus cajan*, early maturity, genomics, grow-out test, hybrids, seed purity

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is the only pulse crop where substantial (25% or more) natural out-crossing occurs (Saxena *et al.*, 2016). Although this phenomenon in pigeonpea was first discovered in 1916, but none of the breeders attempted to use it for genetic enhancement of the crop. It was in 1974 when the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the Indian Council of Agricultural Research (ICAR) launched a joint programme to exploit the natural cross-pollination by developing hybrid cultivars. This endeavour got wings when cytoplasmic nuclear male sterility (CMS) systems were bred by integrating pigeonpea nuclear genome in to the cytoplasm of wild species (Tikka *et al.*, 1997; Saxena *et al.*, 2005). After decades of intensive research, a potential hybrid breeding technology was developed and three medium maturing (175±10 d) hybrids were released in India (Saxena *et al.*, 2013; 2016; 2021a). These hybrids performed well in farmers' fields and, on average, produced 30-50 % higher yields over the local control cultivars. Besides high yields, these hybrids also exhibited greater resilience to various yield-reducing stresses to provide stability to their productivity (Saxena, 2015).

The success of any hybrid technology primarily depends on the economics of producing its genetically pure seed in large quantities. To achieve this objective in pigeonpea, a hybrid seed production system was also developed (Saxena, 2006). Its on-farm validation revealed that in one hectare of production plot about 1000 kg of hybrid seed can be harvested clocking a healthy seed-to-seed ratio of over 200. Unfortunately, despite such high-end accomplishments, the hybrids could not reach farmers because the technology failed to attract commercial seed companies due to a single concern of maintaining a high degree of genetic purity. The seed producers felt that the use of pure seeds, isolations and rouging was not sufficient enough to guarantee the genetic purity of hybrid seed. The authors, hereby, open-up this issue again and put forward some potent solutions to create renewed awareness among the seed companies about the new seed testing procedures that would overcome the problems being faced in the existing the seed system. These can possibly bring the hybrid pigeonpea programs back on rails.

GENETIC PURITY OF SEED - THE SOLE CONSTRAINT

The standard seed quality testing procedure

In any commercial seed programme no entrepreneur would like to compromise with the quality aspects of seed. Hence, strict quality control during seed production and processing is essential. To ascertain this, besides following the recommended cultural practices, each hybrid seed lot is examined against the prescribed quality parameters. Further, in order to facilitate marketing, all the seed-related activities are completed well before the commencement of the following crop season. Besides assessing seeds for physical properties, it is also important to evaluate them for purity at the genetic level. The genetic truthfulness of seeds is generally assessed through the standard "Grow-out Test" (GoT). This is a popular field-oriented and reliable procedure that involves collecting the hybrid seed samples from production plots and growing them in the following off-season without losing time. As a follow-up, the hybridity test is done by scoring single plant progenies for the presence of a known dominant marker trait that was inherited from the male parent of a given hybrid. This data, thus generated is used to select/reject a particular hybrid seed lot.

The pigeonpea related issues

Unfortunately, the GoT could not be applied to the three pigeonpea released hybrids. This problem was associated with the inherent issues of late maturity and photo-sensitivity of the hybrids, and these did not permit raising an off-season nursery during the long summer days. Pigeonpea is a short-day plant and its flowering is induced when day light hours are about 10-11 (Saxena *et al*., 2021b). Since the parents of the hybrids are highly photo-sensitive the hybrids cannot flower during the long photo-periods, if sown soon after the harvest. This lacuna turned out to be the major issue in undertaking the GoT procedure. This left pigeonpea breeders with limited seed quality control options such as the use of pure seeds, adequate isolation, strict monitoring and rouging. The on-farm validation of this handicapped seed production technology revealed that these quality control measures were insufficient to always produce seeds with the desired level of genetic purity. Therefore, better alternatives are needed to revive the hybrid pigeonpea research and development.

CAPPING OF MATURITY - A WINNING SOLUTION

Saxena *et al*. (2021b) reported that the genes responsible for lateness in pigeonpea are dominant and they also control flowering responses to extended photo-periods. Wallis *et al.* (1981), Turnbull *et al*. (1981), Wallace *et al*. (1993) and Silim *et al*. (2007) conclusively demonstrated that the earliest flowering pigeonpea genotypes were the least sensitive to photo-period and *vice versa*. Evidently, there is a strong positive linkage between late maturity and photo-period sensitivity and the genes controlling these two traits are pleiotropic in origin (Saxena *et al*., 2021b); and hence, there is no probability of breeding late-maturing photo-period insensitive genotypes in pigeonpea.

The pigeonpea cultivars maturing up to 140 days are considered "early" (Saxena *et al*., 2019) and in the materials this maturity group two consecutive seed-to-seed generations can be taken within a year and that too before the commencement of the next cropping season (Saxena *et al*., 2018a). With this knowledge and experience, it is apparent that in early maturing pigeonpea hybrids the grow-out tests can be performed with ease. A protocol of producing hybrid seed in the main season and conducting a follow - up GoT in the off-season is summarized in Table 1. The hybrid seed production crop of the early maturity group (when sown at the onset of rainy season) will certainly be harvested by the end of November. To conduct the GoT freshly harvested hybrid seeds can be sown any time in December or even earlier; and due to the prevailing short photo-periods, their progenies will flower by February or early March at the most. At this time observations on the presence/absence of the marker-trait in the hybrid progenies can be recorded to determine the level of hybridity in different seeds lots. This schedule will provide 2-3 clear months to complete the follow-up activities related to hybrid seeds processing, marketing etc. The hybrids belonging to the mid-early group will be ready for harvest in the month of December. In this material also the GoT can also be exercised, but without delaying the harvesting and post-harvest operations (Table 1). On the contrary in late-maturing hybrids, the GoT would not be a practical option due to late harvesting of the hybrid crop in the main season and the following long photoperiods.

Phenotypic markers for GoT of early maturing hybrids

For grow-out testing, the truthfulness of hybrid plants is determined by the presence of dominant

Table 1. Protocol for implementing the phenotypic marker-based grow-out test for early maturing pigeonpea hybrids

Maturity Group	Flower (days)	*PS gene present	Mature (days)	Ref. Cultivar	Hybrid seed production		Grow-out test		
					Sown	Harv.	Sown	Flower	Market
Sup. Early	<50	NR	<90	MN 5 MN-8	July	Sept	Oct	Dec/Jan	May-June
Ex. Early	50- 65	*None	110-120	VLA 1 Manak	July	Oct	Nov	Jan/Feb	May-June
Early	70- 85	*PS_1	121-150	UPAS120 Pusa 992	July	Nov	Dec	Feb/Mar	May-June
Mid-early	90-105	*PS_2	151-160	T S 3 BDN 711	July	Dec	Jan	Mar/Apr	May-June
Long	120-125	*PS_3	180-190	ICPH 2740 ICPH 3762	July	Feb	Long days, no GoT		

*Photo –sensitive gene as reported by Saxena *et al.* (2021b); NR= not reported

marker that has been inherited from the male parent to the offspring. Such marker(s) may be visible in the hybrid progenies during vegetative and/or reproductive stage. Some phenotypic markers which can be used in the GoT of pigeonpea hybrids are listed in Table 2. The seed producers can easily score the test hybrid progenies before flowering for the dominant trait such as lanceolate leaves, purple stem, or indeterminate growth habit. Scoring for the traits such as indeterminate plants and normal lanceolate leaves can be done in about 8-10 weeks from sowing. The stem colour, however, is not a very reliable trait as it may be influenced by the intensity and duration of sun light. The dominant colour markers in the reproductive parts include red flowers, purple pods and brown seeds. Among these, "red flower" is considered the best as the plants with this marker can be scored at the emergence of floral buds. For the "brown seed" marker one would need to wait for about two months after the flowering to get the necessary data. This method will be useful in detecting the hybrid plants only if the female parent of the target hybrid carries the alternate recessive alleles. This way, an error-free scoring of hybrid progenies for hybridity can be accomplished.

Raising a post-rainy winter crop of early pigeonpea may not be feasible at high latitude locations due to prevailing low temperatures. In such situations, the GoT nurseries should be grown at the sites located at lower latitudes. This is because the temperatures at the latitudes <20^0 N would be conducive to grow a full post rainy season crop of pigeonpea with ease.

Evidence of heterosis in early maturity group

Significant levels of standard heterosis (superiority over the control cultivar) in pigeonpea have now been demonstrated in a large number of crosses (Saxena *et al*., 1992; Saxena *et al*., 2014; Saxena, 2015; Saxena *et al*., 2018a, b). In most places the hybrid breeding research was carried out in medium and mid-late maturity groups; and information on the standard heterosis in early maturing hybrids is rather scanty. The productivity data of some elite early maturing hybrids in multi-location trials conducted by ICRISAT are summarized in Table 3. The standard heterosis recorded in this group ranged between 18- 54%. Among these ICPH 3363, ICPH 2431, ICPH 2433, ICPH 2438 and ICPH 2439 were found outstanding with significant levels of standard heterosis exhibited. These hybrids deserve commercialization to benefit the farming community. However, to succeed in such endeavours, the productivity levels and seed production technology of the hybrids should be acceptable to seed companies with high grade of reliability, cost effectiveness and high genetic purity.

Table 2. Some phenotypic markers which can be used for conducting grow out test of early maturing hybrids during the off-season

Trait	Dominant marker	Recessive Phenotype	Reference	Approx. time for expression
Growth habit	Indeterminate	Determinate (1*)	Gupta & Kapoor (1991)	<50 days
Leaf shape	Lanceolate	Obcordate (1)	Saxena et al. (2011)	<50 days
Stem colour	Purple	Green (1)	D' Cruz et al. (1974)	??
Flower colour	Red	Yellow (>1)	D' Cruz et al. (1974)	60 days
Pod colour	Purple	Green (>1)	D' Cruz et al. (1974)	70 days
Seed colour	Brown	White (1)	D' Cruz et al. (1974)	110 days

*Number of gene(s) reported by respective author

Table 3. Mean and range of yield and standard heterosis of early maturing hybrids recorded at 25 locations

Hybrid name	Yield (kg/ha)			Gain
	Maximum	Minimum	Mean	%
ICPH 2433	2538	1864	2306	54
ICPH 2438	2722	1570	2127	42
ICPH 2363	2292	1763	2048	36
ICPH 2429	2105	1907	1946	30
ICPH 2431	2186	1400	1919	28
ICPH 2447	2045	1456	1811	21
Control	1758	1204	1502	-

Adapted from (Saxena *et al*., 2014)

Maintainers and restorers available for breeding new early maturing hybrids

In order to breed high-yielding early maturing hybrids, the availability of new male sterility maintainers and fertility restorers is critical. To achieve this, initiatives were taken at ICRISAT and 86 early maturing testers were crossed with an early maturing A_4 CMS line ICPA 2039 (Saxena *et al*., 2014). All the F_1 plants of each cross were examined for their pollen fertility by squashing and drenching their anthers with aceto-carmine solution and counting the stained pollen grains under light microscope.

Those testers which produced all the F_1 plants with 100% pollen sterility were classified as 'maintainers' and those with 100% pollen fertility were identified as 'restorers'. All the selected testers were maintained by selfing using muslin cloth bags. Of the eight maintainers identified (Table 4), seven had determinate (recessive) growth habit while only one was non-determinate. The earliest maturing (106 days) maintainer genotype was ICPL 20171. Besides growth habit, the other recessive trait available amongst the maintainers and could be used as a phenotypic marker is white seed coat colour and this trait is present in four maintainers (ICPLs 86012, 87093, 87102 and 93093).

Screening of the experimental hybrids for pollen fertility yielded 14 early maturing fertility restorers (Table 4). Interestingly, all of them had brown seeds which can be used as a dominant marker for the conducting the GoT in hybrid seed programs. The maturity among the fertility restorers ranged from 112 (ICPL 88039) to 134 (ICPL 161) days. The availability of eight maintainers and 14 restores provides enough opportunities to breeders to launch an early maturing hybrid breeding programme with suitable phenotypic markers that would allow the production of quality hybrid seeds.

Table 4. Early maturing maintainers and restorers with marker (bold) traits

Genotypes	Days to mature	Growth habit	Seed colour
Male sterility maintainers			
ICPL 93093	110	DT (recessive)	W (recessive)
ICPL 87093	120	DT (recessive)	W (recessive)
ICPL 86012	115	DT (recessive)	W (recessive)
ICPL 87102	110	DT (recessive)	W (recessive)
ICPL 85012	110	DT (recessive)	B (dominant)
Pusa Ageti	128	DT (recessive)	B (dominant)
ICPL 20171	106	DT (recessive)	B (dominant)
ICP 14425	119	NDT (dominant)	W (recessive)
Fertility restorers			
ICPL 149	132	NDT	B
ICPL 81-3	117	NDT	B
ICPL 161	134	NDT	B
ICPL 88034	121	NDT	B
ICPL 88039	112	NDT	B
ICPL 86022	120	NDT	B
ICPL 92047	118	NDT	B
ICPL 92045	110	NDT	B
ICPL 93103	110	NDT	B
ICPL 150	133	NDT	B
ICPL 93107	113	NDT	B
ICP 11378	133	NDT	B
ICP 8744	129	NDT	B
ICP 10907	115	NDT	B

DT = determinate, NDT= indeterminate, W= white, B= brown. Adapted from Saxena *et al.* (2014)

One of the early maturing pigeonpea hybrids ICPH 2431 has recently been identified for possible release (AN Tikle, pers. comm.) in Madhya Pradesh. This hybrid was developed by crossing a determinate CMS line ICPA 2039 with an indeterminate fertility restorer line ICPL 149. At Sehore both the A- and B-lines flowered in 80-85 days and the maturity of B-line was achieved in 135-145 days. It was also observed that the R- line took about 90 days for full flowering. The hybrid seed was produced in isolation through natural cross-pollination. The harvesting of mature hybrid (A x R) seeds was done in about 140-150 days from sowing *i.e.* by early December. In this hybrid combination, the contrasting trait between the two parents is growth habit (determinate vs indeterminate) and this trait can be used as hybridity marker for an error-free GoT.

To conduct the GoT, the freshly harvested hybrid seeds can be sown towards the last week of December. The hybrid plants will flower in about 80-90 days. At this time all the hybrid progenies should be examined for their growth habit. In the progenies observations should be recorded for the frequency of indeterminate (dominant marker) and determinate (recessive) plants. In this GoT the plants with indeterminate growth habit

will confirm their true hybridity. Using this protocol, the purity of the hybrid ICPH 2431 can easily be established by March/ April; and this will leave the producers with sufficient time for processing, marketing and distribution of the hybrid seed.

Similarly, an early maturing hybrid IPH 15-03, with average standard heterosis of 28%, was bred by Indian Institute of Pulses Research, Kanpur (26 ^{0}N) and released for cultivation in the North West Plain Zone released (Saxena *et al*., 2020). The seeds of this hybrid can be produced in the rainy season at Kanpur itself, but a winter GoT - crop cannot be grown due to prevailing low temperatures. The freshly harvested seeds of this hybrid can be grown at the locations such as Hyderabad (17 ^{0}N) for conducting the GoT.

Besides GoT, the genetic purity of early maturing hybrids can also be determined using molecular markers. RK Saxena *et al*. (2010) demonstrated that the purity (hybridity) of an early maturing hybrid ICPH 2438 could be assessed using two SSR markers. Bohra *et al*. (2011) further opined that for determining seed purity of such pigeonpea hybrids at commercial level, these DNA markers can be used in multiplexes.

GENOMICS – A SWAYING APPROACH FOR HYBRID SEED QUALITY CONTROL IN PIGEONPEA

Each year over 80% of the entire pigeonpea area in India is sown with medium and late maturing (>180 days) varieties. In general, the cultivated varieties and landraces are low-yielding and to meet the deficit tonnes of pigeonpea is imported annually. For example, in 2016/17 the national production of pigeonpea in India was reasonable (4.6 m tonnes) but, on the increasing consumer demand, another 703,540 tonnes of grains was imported from Myanmar and Africa (Reuters News Agency, August 5, 2017). To reduce this import burden and meet the challenges of land limitation, population growth and low productivity, there is no way out except to increase the in-country production of this pulse. Further, in the backdrop of decades of yield stagnation, it is believed that in pigeonpea only hybrid technology holds the promise (Saxena and Tikle, 2015). The hybrids, although exhibited significant yield gains, but their commercialization is on hold due to the issues related to seed quality control. After a long wait, a way out to this impasse has now emerged from genomics laboratories.

The genomics technology involves SSR (simple sequence repeat) and SNP (single nucleotide polymorphic) markers. In this context, it is important that the markers selected for hybrid seed testing should be polymorphic with high-quality allelic peaks/bands/calls between A- and R- lines. Such markers can be used to screen DNAs of the hybrid seed or plants along with that of A- and R- lines for use in a given hybrid. In the case a particular seed or seedling shows the two clear fragments (alleles), one each from A- line and R- line, and then such an individual will be tagged as a true hybrid. An efficient genomics-based seed testing protocol, developed by ICRISAT and ICAR, involving molecular markers is now available and it provides the most reliable and rapid results.

Purity assessment of A_4 CMS lines

For assessing the genetic purity of A_4 CMS lines, the genomic identification of specific *Cajanus cajanifolius* cytoplasm as well as the nuclear genome of its maintainer (B-) line should be done. In order to differentiate between A- and B- lines at the molecular level, a gene-based marker (*nad7a_del*) is now available for use. This marker was identified from *nad7* gene that is located in its mitochondria (Sinha *et al*., 2015).

Purity assessment of fertility restorers and hybrids

Initial efforts in this endeavour were focused on the development and use of low throughput SSR markers (RK Saxena *et al*., 2010; Bohra *et al*., 2011). Subsequently, with the availability of draft genome sequence (Varshney *et al*., 2012) and whole-genome sequence data on hundreds of pigeonpea lines (Varshney *et al*., 2012, RK Saxena *et al*., 2021) the focus was shifted towards the development and use of high throughput SNP markers and flexible genotyping platforms for ease in seed testing.

To carry out this activity, diagnostic SNPs markers are now available for A_4 specific fertility restorers 25 pigeonpea hybrid combinations from different maturity groups (RK Saxena, pers. comm.). These SNPs have been converted to customized and a cost-effective Kompetitive allele specific PCR (KASP) genotyping assay. In case the high throughput genotyping facilities are not available in-house, the leaf samples from hybrids and their parents can be supplied to any recognized genomics laboratory for SNP-based diagnostic purposes. The turnover time to get the results is about two weeks. The estimates worked out at ICRISAT showed that for each sample the cost of DNA isolation and genotyping using KASP approach (10 markers data with a minimum of 384 total samples) would be about 2.5 US$.

The above mentioned SNPs - based purity

assessments kits are being used to determine the seed purity of hybrids at the commercial level (RK Saxena, pers. comm.). To produce high-quality seeds of pigeonpea hybrids using genomics tools there is a need to integrate various field and genomics-related laboratory activities. It is important that seeds of the given hybrid and its parents should be produced according to the recommended guidelines with respect to selection of production site, isolation distance, insect management, rouging and other field operations. For laboratory assessment, mature seeds samples should be drawn from each lot as per the prescribed recommendations. This should follow their physical inspection for different parameters, and the cleared samples should be submitted to the genomics laboratory for genomic analysis.

CONCLUSIONS

The traditional pigeonpea genotypes have strict short day photo-period requirement to flower and, irrespective of sowing time, they flower only during the shortening daylights. Following nature's this rule, the freshly harvested seeds of long-duration hybrids, if sown immediately after harvesting (in March/ April) for GoT, will remain vegetative for a long period and their flowering will commence only in the following November month. Therefore, the hybrids of the late maturing group of maturity will always be deprived of the GoT option in their seed production endeavours.

The authors visualize that, in spite of this inherent limitation, the farmers can still reap the benefits of hybrid technology if the breeders opt for early maturity hybrids and/or utilize the latest molecular tools. In the early group hybrids also significant hybrid vigour is available and quality determination through GoT is possible. For the longer version of hybrids the quality assessment can be done using specific molecular markers for which effective protocols are now ready for use. To implement this, it should be noted that (i) for generating the markers the seed should be obtained from a highly reliable source, (ii) the markers should be polymorphic between A- and R- lines and (iii) for a given hybrid, only the proven cross-specific genetic markers should be used.

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Variability studies on pigeonpea sterility mosaic emaraviruses in Tamil Nadu reveals rampant mixed infections and interspecies recombination

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ABSTRACT

Sterility Mosaic Disease (SMD) is one of the most important diseases of pigeonpea in the Indian subcontinent. SMD of pigeonpea is associated with two distinct emaravirus species, *Pigeonpea sterility mosaic emaravirus 1* (PPSMV-1) and *Pigeonpea sterility mosaic emaravirus 2* (PPSMV-2). Both the emaravirus species consist of six negative-sense RNA segments referred to as RNA1, RNA2, RNA3, RNA4, RNA5 and RNA6. In this study, we analysed the variability among 12 isolates of PPSMV from 12 locations of major pigeonpea growing regions of the south Indian state Tamil Nadu. Reverse Transcription -Polymerase Chain Reaction (RT-PCR) analysis showed that both PPSMV-1 and PPSMV-2 were present in all the 12 locations as mixed infections and the segment RNA6 was present in all the isolates of PPSMV-1 and -2. Phylogenetic analysis with the nucleotide sequence of Nucleocapsid Protein (NP) and Movement Protein (MP) revealed that PPSMV-1 is more closely related to *Redbud yellow ringspot virus* than PPSMV-2. Whereas, PPSMV-2 is more closely related to *Fig mosaic virus* than PPSMV-1. Sequence identity analysis of the RNA1 segment of PPSMV-1 isolates revealed that the lowest sequence identity of 83.2% was with the Bihar isolate. Recombination analysis of NP sequences revealed that the Coimbatore-5 isolate of PPSMV-1 showed inter-species recombination with Bidar isolate of PPSMV-1 and Patancheru isolate of PPSMV-2. Likewise, the MP sequence of Trichy-1 isolate of PPSMV-1 showed inter-species recombination with PPSMV-1 Mahagaon isolate and *Raspberry leaf blotch virus* isolate (NCBI Acc. No. FR823301).

Key words: *Emaravirus*, Pigeonpea, Recombination, Variability

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] also referred to as red gram or arhar is a perennial shrub with its centre of origin in India. About 15 plant viruses are reported to naturally infect pigeonpea (Kumar *et al*., 2008) and the sterility mosaic disease (SMD) caused by Pigeonpea sterility mosaic emaraviruses (PPSMVs) is the economically most important viral disease in India. SMD was first reported in 1931 from Pusa, Bihar (Mitra, 1931) and is mostly endemic to India, Nepal, Bangladesh and Myanmar. The eriophyid mite, *Aceria cajani* Channabasavanna is the vector, that transmits this emaravirus in a semi-persistent manner (Kumar *et al*., 2002, 2003).

Two emaravirus species, *Pigeonpea sterility mosaic emaravirus 1* (PPSMV-1) and *Pigeonpea sterility mosaic emaravirus 2* (PPSMV-2) are associated with SMD of pigeonpea, having multiple negative-sense single-stranded RNA segments as their genome (Elbeaino *et al*., 2015). Complete to partial sterility of flowering, stunting, chlorotic rings or mosaic symptoms on the leaves and a reduction in leaf size are the characteristic symptoms of SMD. The nature and severity of symptoms are largely dependent on the pigeonpea genotype and age of the crop at the time of virus infection (Jones *et al*., 2004).

Both PPSMV-1 and PPSMV-2 contain six genomic RNA segments of size 7022 nt, 2223 nt, 1442 nt, 1563 nt,1689 nt and 1194 nt coding for RNA-dependent RNA polymerase (RdRp), glycoprotein (GP), nucleocapsid protein (NP), movement protein (MP), respectively and the segments RNA5 and RNA6 encode for proteins of unknown function (Elbeaino *et al*., 2013, 2014, 2015, Patil *et al*., 2017). The first four RNA segments of PPSMV-2 share higher sequence similarity with FMV than with PPSMV-1 (Patil *et al*., 2017).

The other definitive members of the genus *Emaravirus* reported till date are *European mountain ash ringspot-associated emaravirus* (EMARaV), *Fig mosaic emaravirus* (FMV), *Raspberry leaf blotch emaravirus* (RLBV), *Rose rosette emaravirus* (RRV), *Redbud yellow*

ringspot associated emaravirus (RYRSaV), *Actinidia chlorotic ringspot-associated emaravirus* (AcCRaV) and *High Plains wheat mosaic emaravirus* (HPWMoV) (Patil and Kumar, 2015, 2017, Bello *et al.,* 2015).

To develop any virus control strategy such as RNA-interference or double-stranded (ds)-RNA based, it is important to understand the population structure of the viruses, for which sequence variability studies are essential (Patil *et al*., 2011, Patil *et al*., 2021). In this study, we investigated the current variability status of PPSMV-1 and -2 isolates in Tamil Nadu state of India (Baskar *et al*., 2020). We analyzed all the RNA segments of 12 isolates of PPSMV, collected from 12 locations representing 4 major pigeonpea cultivating districts of the Tamil Nadu state. Further, we subjected these sequences to recombination and phylogenetic analyses and confirmed the presence of RNA6 in all the PPSMV-1 and -2 isolates.

MATERIAL AND METHODS

SMD infected pigeonpea leaf sample collection

Leaf samples with typical SMD symptoms were collected from 12 locations of pigeonpea cultivating regions of Tamil Nadu state, during *Kharif,* 2019 (August - October). Infected leaf samples showing characteristic symptoms like severe mosaic, ringspot, leaf size reduction and stunted growth were collected. The locations were Tamil Nadu Agricultural University (TNAU, Coimbatore) pulse research farm, Devarayapuram and Thondamuthur villages in Coimbatore district, Elur, Perumpallipatti and Kandipalayam villages in Namakkal district, Attaiyampatti, Papparapatti and Minnakkal villages in Salem district and Arachi, Keelakkunnuppatti villages in Trichy district of the Tamil Nadu state (Figure 1) (Baskar *et al.,* 2020). All these leaf samples were immediately brought to TNAU Coimbatore and snap-frozen in liquid nitrogen and stored in an ultra-low deep freezer (-80°C) for further use.

RNA extraction and RT-PCR for the amplification of RNA segments of PPSMV-1 and 2

About 100 mg of symptomatic and healthy pigeonpea leaf tissues were used for total RNA extraction, by crushing them in liquid nitrogen. Total RNA was extracted from leaf powder using Trizol method. The total RNA was reverse transcribed using a cDNA synthesis kit (Verso cDNA Synthesis kit, thermos Fisher Scientific India) with RT Random primers following the manufacturer's instruction. 3 to 4 µg of RNA has been used for cDNA conversion. The cDNA was used as a template for PCR amplification

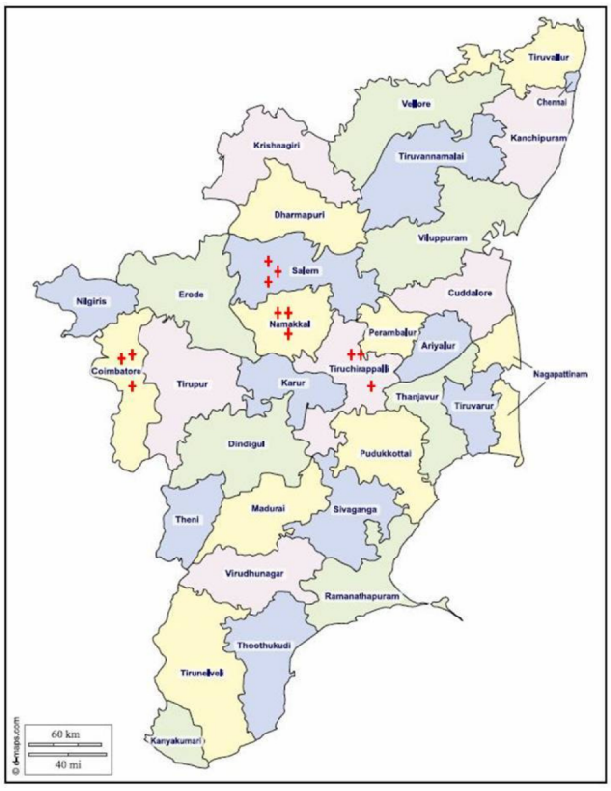

Figure 1. Map of Tamil Nadu state (India) showing the 12 collection sites (+) of the SMD affected pigeonpea leaf samples that were used in this study.

of various RNA segments of PPSMV-1 and PPSMV-2 isolates using the specific primers (Table 1) described in Patil *et al.,* 2017.

Sequence analysis of RNA segments of PPSMV- 1 and PPSMV-2 Isolates

The nucleotide homology search was done by the BLASTN sequence analysis tool of NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The PPSMV sequences were submitted to NCBI GeneBank and accession Numbers were obtained for all the segments of PPSMV-1 and PPSMV-2 isolates. Nucleotide sequences of the segments of PPSMV-1 and PPSMV-2 isolates from this study along with other published sequences of PPSMV-1, PPSMV-2 and selected *Emaravirus* sequences from the NCBI database were aligned using the MUSCLE program and the phylogenetic trees were constructed by maximum likelihood applying Neighbour-joining Method and pairwise gap deletion option inbuilt in the MEGA 6 software. The sequence identity analysis program BioEdit was used to calculate sequence identities and identity matrixes were drawn for the partial nucleotide sequences of RdRp, NP MP and RNA6 of PPSMV-1 and PPSMV-2 isolates.

Table 1. Primers used for RT-PCR amplification of different RNA segments of PPSMV-1 and PPSMV-2 isolates.

Primer Name	Primer Sequence (5′-3′)	Target RNA Segment
PPSMV1-RNA1F	CATTGTATAACACTAAATGAAAN	RNA-1
PPSMV1-RNA1R	CTAACATTCGATTCATTAGCTN	RNA-1
PPSMV1- RNA3-F	CACCATGCCTCCAAAGATGCN	RNA-3
PPSMV1- RNA3-R	TTACTCCTTTAAAGATTTCN	RNA-3
PPSMV2- RNA3-F	CACCATGCCTCCAAAGAGATCAATN	RNA-3
PPSMV2- RNA3-R	CTAAGGCAAGCTAGCCAGAN	RNA-3
PPSMV1- RNA4-F	CACCATGCACGTTTTCCTATTTTTN	RNA-4
PPSMV1- RNA4-R	TTAGGTAGCTTCACCAATTTTTN	RNA-4
PPSMV2- RNA4-F	CACCATGATGCCTAGCACCTCN	RNA-4
PPSMV2- RNA4-R	TTACTGAGCTTCACCTATTAC	RNA-4
PPSMV2- RNA6-F	CACCATGGCGTCAAAGGGATTTGN	RNA-6
PPSMV2- RNA6-R	TCACTCAAGTTGTGATGGTGAN	RNA-6

The recombination detection program package RDP4 was used for the detection of recombination and identification of likely parent sequences. RDP, GENECONV, BOOTSCAN, MAXIMUM CHI SQUARE, CHIMAERA, SISTER SCAN, PHYLPRO and 3SEQ which are the inbuilt methods of the RDP4 program were used to identify the recombination breakpoints. We subjected all the partial sequences of the genomic RNA segments of PPSMV-1 and PPSMV-2 isolates along with FMV, EMARaV, RYRV, RLBV and RRV sequences for RDP analysis. The analyses were done with default settings and with a Bonferroni correction p-value cut-off of 0.05. Recombination events were considered significant only if the p-values were less than 1 $x10^{-6}$ in at least three of the seven methods of the RDP4 package.

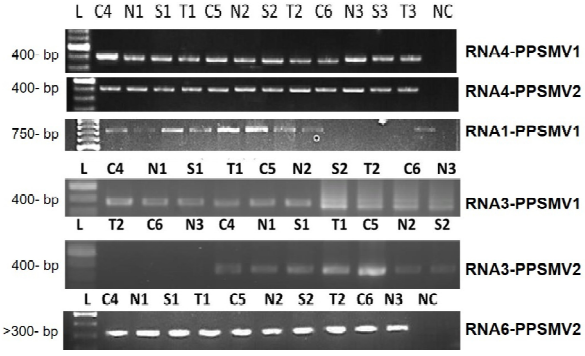

Figure 2. RT-PCR amplification of RNA1, RNA-3, RNA-4 and RNA-6.
Lane L 100 bp DNA ladder; lane C4, C5 and C6 were Coimbatore isolates, lane N1, N2 and N3 were Namakkal isolates, lane S1, S2 and S3 were Salem isolates and T1, T2 and T3 were Trichy isolates. NC-negative control.

RESULTS

RT-PCR for Detection of PPSMV-1 and PPSMV-2

RT-PCR was done for all the 12 isolates by using specific primers for RdRp, NP, MP and RNA-6. All the 12 samples collected from Tamil Nadu state had mixed infections of both PPSMV-1 and PPSMV-2 (Figure 2). Amplified PCR products were sequenced and the sequences were submitted to the NCBI database and accession numbers obtained (Table 2). Nine samples amplified for RNA1 of PPSMV-1, 10 samples amplified for RNA3 of PPSMV-1, 6 samples for PPSMV-2 RNA3, for RNA4 of both PPSMV-1 and -2 all the 12 samples got amplified, and 9 samples amplified for PPSMV-2 RNA6.

Sequence Identity Analysis for RdRp, NP, MP and RNA-6 of PPSMV-1 and PPSMV-2 Isolates

Analysis of percentage nucleotide sequence identity for two RdRp, 11 NP, 23 MP and 8 RNA-6 sequences of both PPSMV-1 and PPSMV-2 isolates along with published PPSMV sequences revealed significant sequence variability. The identity among the RdRp sequences of PPSMV-1 isolates was in the range of 83.2% - 98.5% (Table 3). Sequence identity for NP sequences of PPSMV-1 isolates was in the range of 87.1% - 99.6%. Likewise, sequence identity for MP sequences of PPSMV-1 isolates was in the range of 85.2% - 99% and in the case of PPSMV-2 isolates the sequence identity range was 96.4% - 99%. Likewise, sequence identity for RNA-6 sequences of PPSMV-2 was in the range of 88.3% - 99.4% (Table 4).

Recombination Analysis for PPSMV-1 and PPSMV-2 Isolate Sequences

RDP4 analysis of RNA-1, RNA-3, RNA-4 and RNA-6 sequences indicated the presence of recombinations. RDP4 analysis for RNA-3 sequences showed that the Coimbatore-5 isolate of PPSMV-1 had potential inter-species recombination at 26-251 nt, with Bidar isolate of PPSMV-1 as a major parent and

Table 2. Summary of the 12 isolates of PPSMV-1 and PPSMV-2 and their RNA segments sequenced in this study. The corresponding NCBI accession numbers are given in the parenthesis for each sequence.

Sr. No.		LOCATION	CODE	RNA1	RNA3	RNA4	RNA6
A.	1.	Coimbatore Pulses field of TNAU	C4	PPSMV-1 (MT376945)	PPSMV-1 (MT376947) PPSMV-2 (RT-PCR +ve)	PPSMV-1 (MT376958) PPSMV-2 (MT376966)	PPSMV-2 (MT376970)
	2.	Devarayapuram (CBE)	C5	PPSMV-1 (RT-PCR +ve)	PPSMV-1 (MT376948) PPSMV-2 (RT-PCR +ve)	PPSMV-1 (RT-PCR +ve) PPSMV-2 (RT-PCR +ve)	PPSMV-2 (MT376971)
	3.	Thondamuthur (CBE)	C6		PPSMV-1 (MT376949)	PPSMV-1 (RT-PCR) PPSMV-2 (RT-PCR +ve)	PPSMV-2 (RT-PCR +ve)
B.	4.	Namakkal Perumpallipatti	N1	PPSMV-1 (MT376946)	PPSMV-1 (MT376950) PPSMV-2 (RT-PCR +ve)	PPSMV-1 (MT376959) PPSMV-2 (MT376967)	PPSMV-2 (MT376972)
	5.	Kandipalaiyam (NKL)	N2	PPSMV-1 (RT-PCR +ve)	PPSMV-1 (MT376951) PPSMV-2 (RT-PCR +ve)	PPSMV-1 (MT376960) PPSMV-2 (RT-PCR +ve)	PPSMV-2 (MT376973)
	6.	Namakkal Elur (NKL)	N3		PPSMV-1 (MT376952)	PPSMV-1 (RT-PCR +ve) PPSMV-2 (RT-PCR +ve)	PPSMV-2 (RT-PCR +ve)
C.	7.	Salem Attayampatti	S1	PPSMV-1 (RT-PCR +ve)	PPSMV-1 (MT376953) PPSMV-2 (RT-PCR +ve)	PPSMV-1 (MT376961) PPSMV-2 (MT376968)	PPSMV-2 (MT376974)
	8.	Papparapatti (Salem)	S2	PPSMV-1 (RT-PCR +ve)	PPSMV-1 (MT376954) PPSMV-2 (RT-PCR +ve)	PPSMV-1 (MT376962) PPSMV-2 (RT-PCR +ve)	PPSMV-2 (MT376975)
	9.	Minnakkal (Salem)	S3			PPSMV-1 (RT-PCR +ve) PPSMV-2 (RT-PCR +ve)	
D.	10.	Trichy Arachi A	T1	PPSMV-1 (RT-PCR +ve)	PPSMV-1 (MT376956) PPSMV-2 (RT-PCR +ve)	PPSMV-1 (MT376963) PPSMV-2 (MT376969)	PPSMV-2 (MT376976)
	11.	Keelakkunuppatti (Trichy)	T2	PPSMV-1 (RT-PCR +ve)	PPSMV-1 (MT376957)	PPSMV-1 (MT376964) PPSMV-2 (RT-PCR +ve)	PPSMV-2 (MT376977)
	12.	Arachi B (Trichy)	T3	PPSMV-1 (RT-PCR +ve)		PPSMV-1 (MT376965) PPSMV-2 (RT-PCR +ve)	

Patancheru isolate of PPSMV-2 as a minor parent (Table 5). The RDP4 analysis for RNA-4 sequences showed that Trichy-2 isolate of PPSMV-1 had inter-species recombination at 288-308 nt which had RLBV as a major parent and Mahagaon isolate of PPSMV-1 as a minor parent. The Namakkal-1 isolate of PPSMV-1 had recombination at 8-37nt, with Salem-1 isolate of PPSMV-1 as major parent and an unknown as a minor parent, however, the recombination was significant by only one method. RDP4 analysis for RNA-6 sequences showed that the Coimbatore-4 isolate of PPSMV-2 had inter-species recombination at 26-664

Table 3. Per cent nucleotide sequence identities of RNA-1 RdRp (lower diagonal) of PPSMV-1 isolates.

Seq->	PS1.C4.	PS1.N1.	HF568801.1	PS1.K.	PS1.P.	PS1.PUNE.	PS1.BI.
PS1.C4.	ID						
PS1.N1.	**98.5%**	ID					
HF568801.1	96.7%	96.9%	ID				
PS1.K.	94.9%	95.2%	96.8%	ID			
PS1.P.	94.5%	94.6%	96.7%	97.1%	ID		
PS1.PUNE.	93.8%	94.1%	96.0%	97.9%	96.3%	ID	
PS1.BI.	**83.2%**	83.6%	84.1%	84.5%	83.8%	84.0%	ID

Note: Both highest and lowest percentiles were shown in bold fonts. The PPSMV-1 isolates used in this study are: PPSMV-1 isolates – Coimbatore 4 and Namakkal 1 with PPSMV-1 isolate (NCBI- HF568801.1), Kalaburagi isolate (NCBI- KX363886), Patancheru isolate (NCBI- HF568801), Pune isolate (NCBI- KX363904), Bihar isolate (NCBI- KX363899).

Table 4. Percent nucleotide sequence identities of RNA-6 (lower diagonal) of PPSMV-2 isolates.

Seq->	PS2.C4.	PS2.N2.	PS2.N3.	PS2.S1.	PS2.S2.	PS2.T1.	PS2.T2.	PS2-C.1	PS2-R	PS2-P	FMV
PS2.C4.	ID										
PS2.N2.	96.8%	ID									
PS2.N3.	96.0%	96.7%	ID								
PS2.S1.	97.6%	98.8%	96.1%	ID							
PS2.S2.	97.3%	98.3%	96.0%	**99.4%**	ID						
PS2.T1.	87.8%	**88.3%**	88.7%	87.9%	87.6%	ID					
PS2.T2.	92.5%	93.1%	93.5%	92.7%	92.4%	93.3%	ID				
PS2-C.1	95.6%	97.6%	96.5%	97.1%	96.8%	88.9%	93.6%	ID			
PS2-R	58.7%	59.7%	58.8%	59.6%	59.5%	53.9%	56.8%	60.2%	ID		
PS2-P	93.1%	94.9%	93.9%	94.5%	94.2%	86.3%	91.0%	96.6%	59.8%	ID	
FMV	27.3%	27.3%	27.1%	27.5%	27.6%	23.7%	25.3%	27.0%	41.5%	27.2%	ID

Note: Both highest and lowest percentiles were shown in bold fonts. The PPSMV-2 isolates used in this study were: PPSMV-2 isolates – Coimbatore 4 and 5, Salem 1and 2, Namakkal 2 and 3, Trichy 1 and 2 with Bihar isolate (NCBI- KX363943), Coimbatore isolates (NCBI- KX363944, NCBI- KX363945), Raichur isolate (NCBI- KX363939), Patancheru isolate (NCBI- HG939490) and FMV (NCBI- AB697893).

nt, with PPSMV-2 Patancheru isolate as a major parent and PPSMV-2 Salem-2 isolate as a minor parent and the recombination was significant by only two methods (Table 5).

Phylogenetic Analysis for sequences of PPSMV-1 and PPSMV-2 isolates

Phylogenetic analysis of the RNA-1 sequences of two isolates from this study, namely, Coimbatore 4 and Namakkal 1, showed clustering of these two isolates with other PPSMV-1isolates (NCBI Acc. No. HF568801.1) (Figure 3). All the 11 RNA-3 sequences obtained were PPSMV-1 and were subjected to phylogenetic analysis with selected emaravirus sequences. Isolates of PPSMV-1 and PPSMV-2 formed two separate and distinct clusters. The RNA-3 sequence of FMV clustered with isolates of PPSMV-2. The Raichur and Patancheru isolate sequences of PPSMV-2 were distinctly separated from all the other sub-clusters of PPSMV-2. All PPSMV-2 isolate sequences from Bengaluru clustered together. For PPSMV-1 all 11 isolates from the Tamil Nadu state clustered together. The RNA-4 nucleotide sequences of PPSMV-1 from Coimbatore-4 and Namakkal-1 isolates clustered with other PPSMV-1 isolates. All other PPSMV-1 isolates of Tamil Nadu state distinctly separated out from all the other sub-clusters of PPSMV-1 (Figure 3). All the RNA-6 nucleotide sequences of PPSMV-2 clustered together except the Coimbatore 5 isolate.

DISCUSSION

The major objective of our study was to investigate the diversity of PPSMV isolates, across its genome, in a selected geographical region of southern India. Hence, we selected the Central part of the Tamil Nadu state, where pigeonpea is widely cultivated. The study described here, has for the first time, analysed the diversity of emaraviruses associated with SMD of pigeonpea in Tamil Nadu state. This study shows that PPSMV-1 and PPSMV-2 are widespread across Tamil Nadu state with mixed infections of PPSMV-1 and -2 in contrast to previous reports (Patil *et al.*, 2017).

Emaravirus is one of the most recently established plant virus genera and is taxonomically placed in the newly created family *Fimoviridae*, in the order *Bunyavirales* (Ehret and Muhlbach, 2012). In recent years, there have been increased reports of previously

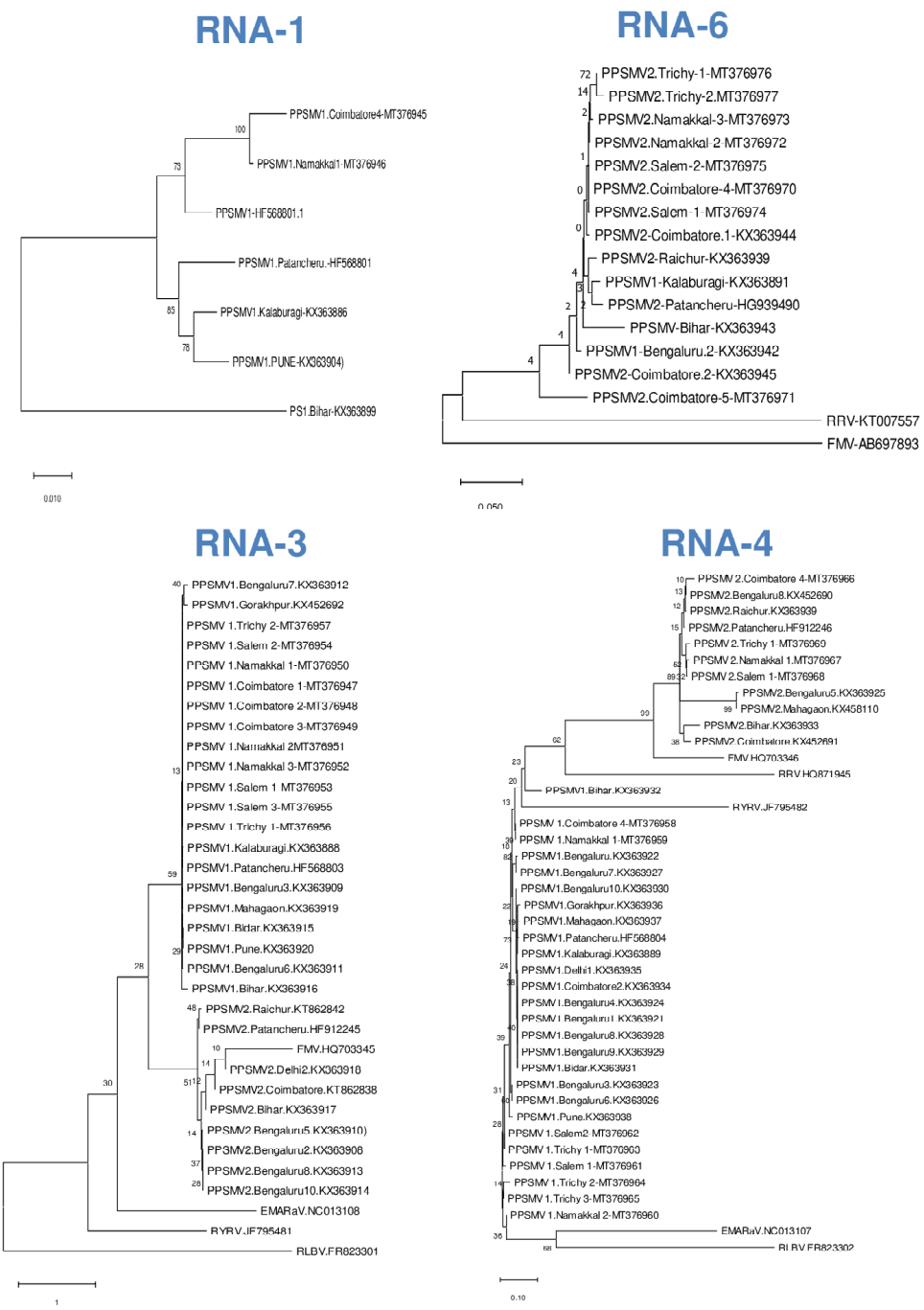

Figure 3. Phylogenetic analysis of the nucleotide sequences of RNA1, RNA3, RNA4 and RNA6.
Note: The nucleotide sequences of the nucleocapsid protein (NP) encoded by RNA3, the putative movement protein (MP) encoded by RNA4, RNA dependent RNA polymerase (RdRp) encoded by RNA1 and protein with the unknown function was RNA6 of PPSMV-1 and PPSMV-2 along with the corresponding sequences of selected emaraviruses, such as *European mountain ash ringspot associated emaravirus* (EMARaV), *Fig mosaic emaravirus* (FMV), *Raspberry leaf blotch emaravirus* (RLBV), *Rose rosette emaravirus* (RRV) and *Redbud yellow ringspot emaravirus* (RYRV). The GenBank accession number for each sequence is given in parentheses. Sequence alignments were done using ''MUSCLE'' and the phylogenetic trees were drawn by the maximum likelihood, applying the JTT matrix and pairwise gap deletion options implemented in MEGA6, using 1000 bootstrap replicates. The scale bar represents 0.1, 1, 0.01 and 0.05 substitutions per nucleotide position for RNA3 and RNA4 sequences, respectively.

Table 5. Summary of unique recombination breakpoints in the RNA segments of PPSMV-1 and PPSMV-2 isolates from Tamil Nadu state as detected by the Recombination Detection Program v.4.16 (RDP4), using seven different methods RDP, GENECONV, BOOTSCAN, MAXIMUM CHISQUARE, CHIMAERA, SISCAN, 3 SEQ. The p-values of all the seven methods are given.

PPSMV Segment		**RNA 3**	**RNA 4**		**RNA 6**
Recombinant Isolate		PPSMV-1 Coimbatore 5	PPSMV-1 Namakkal 1	PPSMV-1 Trichy 2	PPSMV-2 Coimbatore 4
Recombination Breakpoint		26 – 251 nt	08 – 37 nt	288 – 308 nt	26 -664 nt
Parent Isolates	Major	PPSMV-1 Bidar	PPSMV-1 Salem 1	RLBV	PPSMV-2 Patancheru
	Minor	PPSMV-2 Patancheru	Unknown	PPSMV-1 Mahagaon	PPSMV-2 Salem 2
p-values for 7 recombination detection methods of RDP4	RDP	3.97×10^{-02}	NS	4.21×10^{-07}	2.07×10^{-06}
	GENECONV	1.69×10^{-07}	1.02×10^{-02}	3.88×10^{-06}	2.92×10^{-10}
	BOOTSCAN	NS	NS	NS	NS
	MAXIMUM CHISQUARE	3.51×10^{-06}	8.44×10^{-07}	6.45×10^{-06}	1.07×10^{-04}
	CHIMAERA	1.52×10^{-08}	NS	4.33×10^{-04}	1.5×10^{-04}
	SISCAN	NS	NS	NS	NS
	PHYLPRO	NS	NS	NS	NS
	3 SEQ	NS	NS	2.41×10^{-08}	2.19×10^{-02}

Non-Significant p-values are indicated as NS. Parents of the interspecies recombination are marked in bold fonts

unidentified emaravirus species and their genomic RNA segments. Additional genomic segments including RNA5 and RNA6 of FMV and RRV (Babu *et al.*, 2016, Elbeaino *et al.*, 2012) and RNA4, RNA5 and RNA6 of RLBV have recently been reported (Ishikawa *et al.*, 2012). In our study all the isolates of PPSMV-2 from Tamil Nadu state had RNA6, which is similar to previous reports by Elbeaino *et al.*, 2015 and Patil *et al.*, 2017. The diagnostic RT-PCR analyses prove mixed infections of PPSMV-1 and PPSMV-2 in pigeonpea. Recombination breakpoints were detected for RNA-3, RNA-4 and RNA-6 segments, but not for RNA1 and these results were similar to previous reports (Patil *et al.*, 2017). This is the first report of inter-species recombination in RNA4 of two distinct emaravirus species infecting two different crops, namely, RLBV and PPSMV-1, infecting two different plant species. Patil *et al.*, (2017) had reported interspecies recombination between PPSMV-1 and PPSMV-2, but both the species had pigeonpea as their common host.

These studies showed evidence for the exchange of RNA-3 segment from PPSMV-1 to PPSMV-2. Similarly, such segment reassortments were reported for RNA-4 (Patil *et al.*, 2017) and FMV (Walia *et al.*, 2014). Reassortment of viral RNA segments can provide fitness advantages to the progeny viruses or reduce the fitness of a virus depending on the gain or loss of the beneficial alleles (McDonald *et al.*, 2016, Patil *et al.*, 2017).

Taken together, this study is the first attempt to analyse the sequence variability of PPSMV-1 and PPSMV-2 isolates in Tamil Nadu state, which also provides the evidence for recombination and reassortment among their genomic segments. The possibility of mixed infection of PPSMV-1 and PPSMV-2 and inter-species recombination was also revealed. Information on the distribution of PPSMV-1 and PPSMV-2 in Tamil Nadu state will be of significant importance in developing diagnostic tools for the detection of PPSMV-1 and PPSMV-2 isolates and also for the development of sound SMD management strategies in pigeonpea (Patil *et al.*, 2020).

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Host plant resistance and epidemiology of sterility mosaic virus disease in pigeonpea (*Cajanus cajan* (L.) Millsp.)

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ABSTRACT

Field experiments were conducted to identify the resistant sources for Sterility Mosaic Disease (SMD) in pigeonpea at the experimental farm, Department of Pulses,Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University,Coimbatore. Out of 25 genotypes screened under field condition by infector row technique, three entries *viz*., BDN 2, IPA 8F and MA6 showed resistant reaction to SMD consistently for three years with the mean disease incidence of 8.3, 6.9 and 8.7 % respectively. Seven genotypes viz., BRG1, BRG3, BSMR 736, ICP 7035, ICP 2376, IPA 15F and KPL 44 were categorized as moderately resistant genotypes with the disease incidence ranging from 14.8 - 19.2 %. Six genotypes exhibited moderately susceptible reaction, seven genotypes were susceptible and the remaining two genotypes were highly susceptible to the disease. The susceptible checks *viz*., CO5 and ICP8863 recorded the SMD incidence of 85.3 and 93.6 % respectively. All the 25 pigeonpea genotypes were also evaluated under glass house for their reaction against SMD by leaf stapler technique. The genotypes, *viz*., BDN 2, IPA 8F and MA6 also exhibited resistance to SMD under artificial inoculation condition, whereas the susceptible checks *viz*., CO5 and ICP 8863 recorded 100 % SMD incidence. Wide variations were found between resistant and susceptible pigeonpea genotypes for SMD symptom expressions. The resistant genotypes would be of great value for development of pigeonpea cultivars with SMD resistance. In the present study, results of the experiment on epidemiology of SMD indicated that SMD incidence and mite population were negatively correlated with temperature and positively correlated with relative humidity. The average temperature of 29 - 29.2° C and the RH of 89 - 92.5 % was found to favour the SMD incidence in pigeonpea. The results from this study would be helpful to take timely decision for management of SMD.

***Key words*:** Epidemiology, Genotype screening, Pigeonpea, Resistance, SMD

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millsp.), also known as redgram or arhar, is the fifth prominent pulse crop in the world and the second most important pulse crop in India. It is one of the high value and low input requiring drought tolerant pulse crops that offers many benefits to farmers as food, fodder, feed and fuel. It is widely grown in Tamil Nadu as a rainfed crop. Globally, pigeonpea cultivation is spread over an area of 7.02 m ha with an average production and productivity of 6.81 m tonnes and 970 kg ha^{-1} respectively (FAOSTAT, 2017). In India it is grown in an area of 4.78 m ha with the production of 3.59 m tonnes and productivity of 751 kg ha^{-1} (DES 2018). India stands first in the area and production of pigeonpea in the globe but its productivity lower than world average (FAOSTAT, 2013). The biotic and abiotic factors encountered by crop at different stages of growth are majorly responsible for this yield gap in India. Among biotic factors, Sterility Mosaic Disease (SMD) incited by Pigeonpea Sterility Mosaic Virus (PPSMV) is an important constraint which is found to occur in almost all pigeonpea growing regions. The infected plants show bushy and pale green appearance with small leaf, excessive branches, partial or complete sterility, sometimes part of the plants show symptoms other parts remain normal (Kumar *et al.,*2003).The disease is transmitted through an Eriopphid mite (*Aceria cajani* Channabasavanna) in a semi persistent manner (Kulkarni *et al.*2002; Jones *et al.,* 2004).

The yield loss caused by SMD was estimated up to 95 % and it depends on growth stage of the plant at which infection occurred (Kannaiyan *et al*. 1984; Ganapathy *et al.,* 2011). Early stage of infection resulted in 90 % yield loss in pigeonpea (Bhaskaran and Muthiah, 2005). Management of SMD through

acaricides is not much effective and economic, moreover it causes environmental pollution. Exploiting host plant resistance is the most viable and economic strategy for SMD management. Developing resistant varieties in pigeonpea is a difficult task because of genetic plasticity of PPSMV whose virulence depends on location-specific environments (Sharma *et al*. 2012b). Reddy *et al.* (1993) reported existence of five different isolates of PPSMV in India. The variation in SMD symptom expression have been observed among various pigeonpea genotypes and it differs based on time of infection (Ghanekar,1992; Reddy *et al*.,1993). Though SMD is widely prevalent in most of pigeonpea growing regions, its incidence varies seasonally and also from one region to another (Kumar *et al*., 2008). Progress of development of SMD depends on proximity to source of inoculum, plant age, pigeonpea cultivar, climatic factors and mite population (Teifion Jones *et al*., 2004). In India, limited research work has been done on epidemiology of SMD. Many workers identified resistance sources against SMD across the world but host resistance in disease management is seriously curtailed as a result of genetic breakdown or change in virulence of pathogen, making it imperative to continuously search for resistant sources. Therefore, the present investigation was carried out with the objective of identifying resistant sources for SMD and to ascertain the influence of .climatic factors *viz*., temperature, RH and wind velocity on SMD incidence.

MATERIALS AND METHODS

Evaluation of pigeonpea genotypes for SMD resistance

Experimental site and source of seeds

Field experiments were conducted at the Research farm, Department of Pulses, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore (11.0168°N, 76.9558°E) consecutively for the three years during 2015 - 2018, *kharif* season to identify the resistant sources for SMD. The experimental material comprising of 25 genotypes and susceptible check ICP 8863 was obtained every year from AICRP pigeonpea coordinating centers. Seeds of local check variety *viz*., CO5 was collected from the Department of Pulses, TNAU, Coimbatore. The ten genotypes used for studying the symptom variability was also received from pigeonpea AICRP coordinating centers.

Field Experiments (Infector row technique)

The seeds of 25 test genotypes were sown during the first week of August in 4 m row with spacing of 75 cm and plant to plant to plant spacing of 20 cm. Two replications were maintained for each genotype. The local susceptible check CO5 was raised in between 4 rows of test genotypes. The National susceptible check ICP 8863 was sown along border lines of test rows to increase disease pressure. The crop was maintained by following standard agronomic practices as per the recommendation of the TNAU crop production guide without spraying any insecticides or fungicides. The observations recorded on SMD incidence on 30, 60, 90, 120 and 150 days after sowing and the percent disease incidence were calculated using the formula

$$\% \text{ SMD incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

Based on the % disease incidence the genotypes were categorized for SMD resistance by adopting disease score developed by Pande *et al.* (2012) with slight modifications

SMD incidence %	Disease reaction
0 – 10	Resistant
10.1 – 20	Moderately resistant
20.1- 30	Moderately Susceptible
30.1- 50	Susceptible
50.1 – 100	Highly Susceptible

Glass house Evaluation (Leaf stapler technique)

All the 25 genotypes evaluated under field were also tested under glass house for their resistance against SMD under glass house by leaf stapler technique. The seeds of test genotypes were sown in 30 cm pots containing mixture of red soil + sand + FYM (2:1:1) @ five seeds/ pot. Each genotypes four replications were maintained. The inoculum source *viz.*, SMD infected leaflets were collected from the ICP 8863 which was maintained at the PL480 glass house, Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore. The leaflets were observed under the Binocular microscope for the presence of Eriophyid mite (*A. cajani*). The infected leaf lets with mite was stapled on the 10 - 12 days old seedlings of each test genotype in such a way that the lower surface of the leaflets were in contact with the both the leaf surface of the seedling. The variety CO5 and the ICP 8863 were used as the susceptible checks. The observation on SMD incidence was recorded on 30, 45 and 60 days after sowing and the reaction of the genotypes against SMD was determined as per the disease score described above.

Influence of climatic factors on mite population and SMD incidence:

The influence of weather factors *viz*., temperature, relative humidity (RH) and wind velocity on SMD

incidence and mite population was determined by conducting field experiments consecutively for three years from 2015-16, 2016-17 and 2017-18 using susceptible check ICP 8863. Every year, early, normal and delayed sowing was taken during the third week of July, first week of August and third week of August respectively. For each sowing, 30 rows of 4 m length with plant to plant spacing of 20 cm were maintained. The plants were maintained by adopting standard package of practices without taking any plant protection measures. The observations recorded on SMD incidence at 15 days intervals starting from second week of September to second week of January. The meteorological observations *viz*., temperature, RH and wind velocity were obtained from the Agro climate Research Centre, Tamil Nadu Agricultural University, Coimbatore. The % disease incidence for SMD was worked out. The Area Under Disease Progress Curve (AUDPC) was calculated as described by Campbell and Madden (1990) and the Rate of spread of disease per day(r) was worked out as per the formula furnished below. The correlation between weather parameters *viz*., temperature, RH and wind velocity and SMD incidence was determined.

$$\text{AUDPC} = \sum_{i-1}^{n-1} \left[\frac{X(i+1) + (Xi)}{2} \right] x \left[t(i+1) - t\,i \right]$$

Where Xi = intensity of disease at i^{th} observation

t_i = time interval

$$\text{Rate of spread of disease(r)} = \frac{X_2 - X_1}{t_2 - t_1}$$

X_2 = disease proportion at time t_2

$t_2 - t_1$ = time interval

The mite population was recorded at 15 days intervals starting from second week of September to second week of January. Five plants were selected and from each plant three trifoliate leaves were collected and the mite population count was directly recorded under stereo- binocular microscope. It was expressed as number of mites per trifoliate leaf.

RESULTS AND DISCUSSION

Reaction of pigeonpea genotypes against SMD

A total of 25 pigeonpea genotypes were evaluated in the field for SMD resistance by infector row technique for three years from 2015 to 2018 and these genotypes revealed varying response against SMD. Amongst these, three genotypes *viz*., BDN 2, IPA 8F and MA6 exhibited resistant reaction to SMD across three years that recorded the mean SMD incidence of 8.3, 6.9 and 8.7 % respectively. SMD incidence in seven genotypes *viz*., BRG 1, BRG 3, BSMR 736, ICP 7035, ICP2376, IPA 15F and KPL 44 ranged between 14.8 - 19.2 % and were grouped as moderately resistant. Six pigeonpea genotypes *viz*., BRG 2, BRG 4, CRG9701, ICP 7119, KPL 43, MAL 13 with the SMD incidence of 24.2 – 29.2 were categorized as moderately susceptible. Seven entries showed susceptible reaction and the two genotypes *viz*., MAL 43 and RVSA 07-31 were highly susceptible to SMD. The susceptible checks *viz*., CO 5 and ICP8863 registered a mean SMD incidence of 85.3 and 93.6 % respectively (Tables 1 and 2). Earlier, several workers identified resistant sources for PSMD. Shiv *et al*. (2008) reported that out of 22 pigeonpea genotypes, TT 701 was completely free from SMD infection. Sharma *et al*. (2015) carried out multi-environment screening and identified broad based stable resistant sources *viz*., ICPL 20094, ICPL 20106, ICPL 20098 and ICPL 20115 against SMD. Out of 60

Table 1. Reaction of pigeonpea genotypes against SMD under field condition (Infector row technique)

S. No.	Pigeonpea genotypes	SMD incidence (%)* 2015-16	2016-17	2017-18	Mean Incidence (%)
1	**BDN 2**	**9.5**	**9.2**	**6.3**	**8.3**
2	BRG 1	23.4	17.7	18.4	19.8
3	BRG 2	19.9	20.7	32.1	24.2
4	BRG 3	15.5	15.0	17.8	16.1
5	BRG 4	34.5	22.5	30.5	29.2
6	BSMR 736	17.4	19.6	19.1	18.7
7	BSMR 853	45.0	42.6	40.5	42.7
8	CO 6	43.5	41.0	38.5	41.0
9	CRG 9701	30.5	28.4	25.7	28.2
10	ICP 7119	31.7	28.4	27.1	29.0
11	ICP 7035	17.5	14.4	12.5	14.8
12	ICP 2376	18.7	20.0	15.9	18.2
13	**IPA 8F**	**7.7**	**6.9**	**6.3**	**6.9**
14	IPA 15 F	24.4	18.2	15.5	19.3
15	JKM 189	41.7	44.2	59.7	48.5
16	KPL 43	24.1	25.5	27.9	26.0
17	KPL 44	20.0	19.3	18.4	19.2
18	MAL 13	21.4	27.4	29.7	26.2
19	MA 6	**9.6**	**9.3**	**7.1**	**8.7**
20	MAL 43	68.7	58.4	59.2	62.1
21	RVSA 07-31	54.6	51.7	48.6	51.6
22	RVSA 07-29	55.8	48.1	45.9	49.3
23	RVSA 07-10	52.5	47.0	48.6	49.3
24	WRGE 65	60.0	55.0	18.4	44.4
25	WRP 1	51.9	40.5	45.	45.8
26	CO 5	87.5	90.3	100	85.3
27	ICP 8863	90.7	92.5	100	93.6

*Mean of two replications

Table 2. Grouping of pigeonpea genotypes based on their reaction against SMD in the field

Pigeonpea genotypes	No. of genotypes	Disease incidence (%)	Disease reaction
BDN 2, IPA 8F ,MA6	3	6.9 - 8.7	Resistant
BRG 1, BRG 3, BSMR 736,ICP 7035 ICP2376, IPA 15F, KPL 44	7	14.8 - 19.2	Moderately resistant
BRG 2, BRG 4, CRG9701, ICP 7119, KPL 43, MAL 13	6	24.2 – 29.2	Moderately Susceptible
BSMR 853, CO6, JKM 189, , RVSA 07-10, RVSA 07-29 WRP 1,WRGE 65	7	41- 49.3	Susceptible
MAL 43 , RVSA 07-31	2	51.6 - 62.1	Highly Susceptible
Local Susceptible Check Co5	1	85.3	Highly Susceptible
National Susceptible Check ICP 8863	1	93.6	Highly Susceptible

pigeonpea genotypes evaluated for SMD resistance eight entries viz., ICPL-87119, ICPL-2376, BDN-2, PT-4-307, CORG-9701, BSMR-736, GRG-811 and BSMR-853 were resistant to SMD (Vijaya Bhaskar, 2016). Prabhavathi and Ramappa (2018) reported that only one entry *viz*., RKPV 405-10 showed resistant reaction to SMD among the 13 genotypes and the remaining were susceptible with 68 to 100 %.

The reaction of all the 25 genotypes against SMD were tested under glass house condition by adopting leaf stapler technique along with susceptible checks CO5 and ICP 8863 .The genotypes *viz*., BDN2, IPA 8F and MA6 were found to be resistant in the artificially inoculated condition also. Four genotypes viz., BRG 3, ICP 7035, ICP2376 and KPL 44 were grouped under moderately resistant category which recorded SMD incidence ranged from 19.3 to 20.0 % (Fig 1**).** Eight genotypes showed moderately resistant reaction, the susceptible checks *viz*., CO5 and ICP8863 recorded 100 % SMD incidence (Fig 2). Manjunatha *et al.*(2013) found that the genotypes *viz*., ICP 7035, BRG3, ICPL87091, GT101 and JKM189 were found to be resistant to SMD. Tharageshwari *et al.*(2019) evaluated 94 genotypes under glass house by leaf stapler technique and found four genotypes *viz*., DPP 2-89, DPP 3-182, IC 22557 and ICP 3666 were resistant to SMD.

Variability in symptom expression for SMD in resistant and susceptible genotypes

The SMD symptoms expressed by the resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible genotypes were studied under field condition by adopting infector row technique. From this study, wide variation was observed between these genotypes for SMD expression. The resistant genotypes *viz*., IPA 8F showed only ring spot and upward cupping of leaves and the moderately resistant genotypes *viz*., BRG1 and BRG3 expressed symptoms *viz*., ring spot, mild chlorosis, stunting and partial sterility. The moderately susceptible and genotypes exhibited all symptoms of SMD except few symptoms while in highly susceptible genotypes *viz*., CO5 and ICP 8863 all the characteristic symptoms of SMD including complete sterility were observed(Table 3). This was in concordance with findings of Ghanekar (1992) who reported that SMD symptom expression in pigeonpea depends on type of genotype. Ssymptoms of SMD varied with location (Reddy *et al*., 1998) and its incidence differed from plant

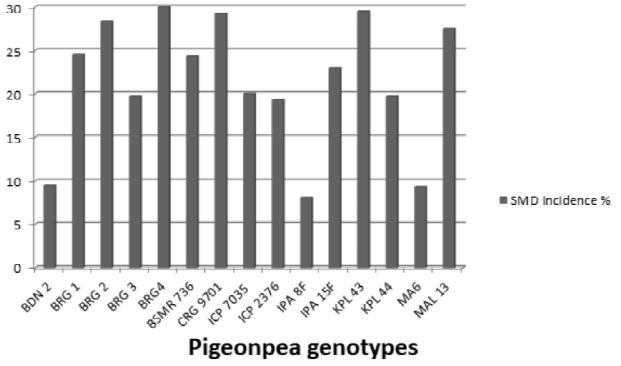

Fig. 1. Incidence of SMD in resistant, moderately resistant and moderately susceptible genotypes identified in glass house evaluation

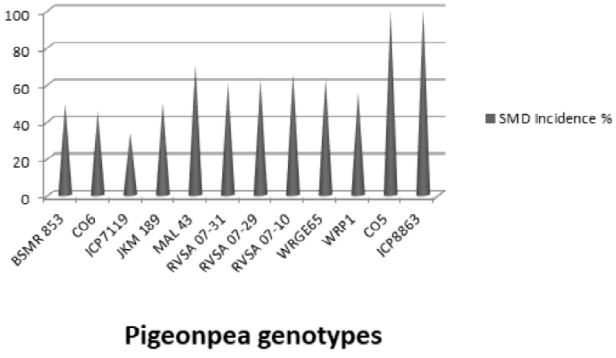

Fig. 2. Incidence of SMD incidence in susceptible and highly susceptible genotypes identified in glass house evaluation

to plant due existence of variability in the pathogen (Kulkarni *et al*., 2003). In SMD resistant varieties, flowering and pod formation was normal while in susceptible variety complete cessation of reproductive parts occurred due to severe mosaic symptoms (Kaushik *et al*., 2013). The variable symptoms expressed by the resistant and susceptible genotypes helped to identify the SMD resistant sources easily.

Epidemiology of SMD

Epidemiological studies revealed that SMD appeared during second week of September in early sown crop (3rd week of July) and gradually reached 100 % incidence during second week of December. However, in the normal sown crop (1st week of August) and delayed sown crop (3rd week of August) crop also 100 % SMD incidence was observed during December second week. The Area Under Disease Progress Curve (AUDPC) showed an increase from initial incidence to later stages of infection and reached to 1466.4, 1432.5 and 1301.3 during the second week of December in early, normal and delayed sowing respectively. The maximum AUDPC of 1500 was recorded in all the three sowing during the fourth week of December (Table 4). The rate of spread of disease per day was the highest (1.3) during the November fourth week in the early sown crop and in normal sown crop the spread was more (1.7) during the second week of October and in late in crop it was high (1.8) during fourth week of October. Ranjit Kumar Paul *et al* (2018) studied

Table 3. Differential symptoms of SMD expressed by various pigeonpea genotypes

S. No	Nature of symptoms	Genotypes									
		CO 5	CO6	CO(Rg) 7	Bahar	BRG 1	BRG 3	SMR 736	IPA 8F	ICP 8863	Purple
1.	Rings pot	+	+	+	+	+	+	+	+	+	-
2.	Mild chlorosis	-	-	-	+	+	+	-	-	-	+
3.	Severe chlorosis	+	+	+	-	-	-	+	-	+	-
4.	Malformation of leaves	+	+	+	-	-	-	-	-	+	-
5.	Puckering of leaves	+	-	-	-	-	-	-	-	+	-
6.	Upward cupping of leaves	-	-	-	+	-	-	-	+	-	-
7.	Reduction in leaf size	+	+	+	-	-	-	-	-	+	+
8.	Bushy appearance of the plants	+	+	+	-	-	-	-	-	+	-
9.	Stunting of plants	+	-	+	-	+	+	-	-	+	+
10.	Partial sterility	+	-	+	-	+	+	-	-	+	
11.	Complete sterility	+	+	+	-	-	-	-	-	+	-
12	Disease reaction	HS	S	MS	MR	MR	MR	MR	R	HS	R

+ Present, - Absent, HS - Highly Susceptible, S - Susceptible, MS - Moderately Susceptible, MR - Moderately Resistant, R - Resistant

Table 4. SMD incidence and mite population in pigeonpea and environmental variables

Time of Observation	Earlier sowing (IIIrd of week of July)*				Normal sowing Ist week of August*				Delayed sowing IIIrd week of August*				Temperature oC*	RH %*	Wind velocity Km/ h*
	Incidence (%)	AUDPC	Rate of spread/ day	Mite population	Incidence (%)	AUDPC	Rate of spread/ day	Mite population	Incidence (%)	AUDPC	Rate of spread/ day	Mite population			
September IInd week	11	-	-	1.5	0	-	-	-	0	-	-	-	32.5	76	6.1
September IVthweek	26	277.5	1.0	3.7	5	37.5	0.3	4.8	0	0	-	-	32.3	90.5	5.1
October IInd week	45	532.5	1.2	15.4	31	270	1.7	17.7	8.5	63.8	0.5	7.2	31.75	85	5.7
October IVthweek	60.5	791.3	1.0	27.3	49.5	603.7	1.2	30.5	36.5	337.5	1.8	25.5	31.9	88.5	4.2
November IInd week	76	1023.7	1.0	31.5	66.5	870.0	1.1	35.7	48.5	637.5	0.8	30.7	30.0	94.5	3.3
November IVthweek	95.5	1286.3	1.3	40.2	91	1181.3	1.6	42.4	73.5	915	1.6	39.1	29.2	92.5	4.9
December IInd week	100	1466.4	0.3	43.7	100	1432.5	0.6	46.7	100	1301.3	1.7	42.6	29.0	90.0	5.8
December IVthweek	100	1500	0	45.5	100	1500	0	52.3	100	1500	0	50.4	29.5	89.0	5.9
January IInd week	100	1500	0	40.5	100	1500	0	50.5	100	1500	0	45.2	29.3	87.0	5.3

*Mean of three years data (2015- 16, 2016- 17 and 2017 – 18)

Table 5. Correlation between SMD incidence, mite population and environmental variables

Details of sowing	SMD incidence %			Mite population		
	Temperature °C	RH %	Wind velocity Km/h	Temperature °C	RH %	Wind velocity Km/h
Early sowing (3rd of week of July)	- 0.953	0.600	- 0.132	- 0.932	0.562	- 0.1176
Normal sowing (1st week of August)	- 0 .956	0.530	- 0.0746	- 0.922	0.552	- 0.122
Delayed sowing (3 rd week of August)	- 0.933	0.426	0.047	- 0.925	0.509	- 0.091

incidence of SMD during *kharif* seasons of 2012-15 and found that the infection occurred during second week of August with peak incidence during third week of October to November.

In the early sown crop the mite population of 1.5 was recorded during the initial crop growth period and it was increased and reached the maximum of 45.5 during the fourth week of September. In the normal and delayed sown crop also the mite population showed an increasing trend from early crop growth to later stage. The highest population of 52.3 and 50.4 was observed in early and late sown crop respectively during the fourth week of December. Pallavi *et al.*(2021) recorded the lowest disease incidence and mite population during the early crop growth period which gradually increased at later stage of crop growth period. The results of the present investigation revealed that, the period between the fourth week of November to second week of December was found to be favorable for SMD incidence and the multiplication of mites. During this period the temperature of 29 to 29.2 °C and RH of 89 - 92. 5 % and the wind velocity of 4.8 – 5.9 km / h were recorded. Pallavi *et al.* (2021) found that the maximum temperature of 27.6 to 38.9°C and RH of 82.4 to 91.3% coupled with scanty rains prevailing during April-June at Bangalore favoured the rapid multiplication of the vector leading to higher disease incidence.

The results of the correlation analysis showed that the SMD incidence and mite population were strong negative correlation with temperature (Table 5). The present finding is in corroboration with the results reported by Reddy and Raju (1993) and Ranjit Kumar Paul *et al* (2018). Kausik *et al* (2013) found that very high temperature is not suitable for mites and the average temperature of 20-30°C was found to be congenial for the multiplication of mite. In this study, the positive correlation was found between SMD incidence and RH. Pallavi *et al* (2021) observed positive influence of morning RH on SMD during June, July, while Ranjit Kumar Paul *et al*. (2018) reported negative correlation of evening RH with SMD. Increased mite population resulted in increased SMD severity (Lakshmikantha and Prabhuswamy, 2002). In the present investigation insignificant correlation was observed between wind velocity and SMD incidence. Reddy *et al*. (1990) reported that SMD can spread up to 2 km downwind from the source of inoculum but the spread in an up-wind direction was very limited (less than 200 m) confirming that wind assist in mite dispersal.

CONCLUSION

The genotypes *viz.*, BDN2, IPA8F and MA6 showing resistance to SMD both under field and glass house conditions. The identified genotypes from this study will be exploited in breeding programme for developing elite pigeonpea cultivar with SMD resistance. In future, detailed epidemiological studies on SMD will be helpful for developing forecasting model to give forewarning to the farmers to take protection measures against the disease.

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Genetic analysis for protein, micronutrients and yield attributing traits in chickpea (*Cicer arietinum* L.)

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ABSTRACT

A study with 24 *desi* chickpea genotypes was taken up at Regional Agricultural Research Station, Nandyal, Andhra Pradesh during *rabi* 2017-18 to get an insight into the existing genetic variability and diversity for protein, iron, zinc, manganese and copper content and also for yield traits. Analysis of variance revealed substantial genetic variability in the genotypes for traits contributing to yield, machine harvestable traits, protein content and micronutrients. High heritability coupled with high genetic advance was noticed for protein, iron, zinc, copper contents and also for yield traits. Phenotypic selection could be successfully be exerted on these for enhancement of traits. High heritability with moderate genetic advance for plant height and low heritability with low genetic advance for manganese content indicated that both additive and non-additive gene action could be governing the traits which in turn results in slow progress through selection for these traits. Mahalanobis D^2 statistic categorized 24 genotypes into seven distinct clusters. Nearly 41.67 % genetic diversity was contributed by height of the first pod followed by number of pods per plant (24.64%). Genetic diversity due to seed yield and plant height was 7.61 and 3.99% respectively. Among nutritive traits, protein has contributed up to 7.97 % followed by zinc (5.8%), copper (3.62%) and iron (2.17%). Some promising genotypes of the present study *viz*., ICC 14402, ICC 1398, ICC 9942 , ICC 6874, ICC 14831 and ICC 7441 which were assorted into diverse clusters hold promise in breeding nutritionally rich chickpea *vis a vis* addressing breeding for high yield and also designing genotypes amenable for machine harvest.

***Key words*:** Chickpea, Micro nutrients, Protein, Seed yield

INTRODUCTION

Grain legumes are also considered as poor man's meat as they play an important dietary role in the diet of millions of people in developing countries. Chickpea (*Cicer arietinum* L.) is one of the most important grain legumes across the globe in terms of production and harvested area. India is global leader in both production and consumption of chickpea with 66 % of total chickpea area and 65 % of production. The cultivable area of chickpea in India is 10.56 million ha with a production of 11.22 million tons with productivity of 1063 kg per hectare (FAOSTAT, 2019). In India, Madhya Pradesh is the largest producer sharing 40 % of total production followed by Maharashtra, Rajasthan, Karnataka, Uttar Pradesh and Andhra Pradesh. Current chickpea breeding programs have mainly been directed towards high yield, biotic and abiotic stress resilience that has increased global production, but less attention has been directed towards improving nutritive worth in seeds. Chickpea, one of the most celebrated vegetarian ingredients in the cuisine, are nutritionally very well endowed and are not only a source of protein, but also rich in dietary fibre, vitamins, and minerals, especially calcium, potassium, iron, zinc, copper (Wallace, 2016). Therefore, characterization and study of existing genetic variability and genetic diversity within the available germplasm for nutritive traits apart from yield traits was taken up with 24 *desi* chickpea genotypes to identify divergent and potential parents for future hybridization programmes for yield and improvement in nutritive worth.

MATERIALS AND METHODS

Field experiment was conducted with twenty four desi chickpea genotypes obtained from ICRISAT during *rabi* 2017-18 at Regional Agricultural Research Station, Nandyal, Andhra Pradesh, India. The genotypes were grown in a Randomized Block Design with three replications. Each genotype was grown in an area of 1.2 m^2 consisting of plots of single row of four meter length with a spacing of 30 cm x 10 cm.

Border crop of chickpea was grown all around experimental area. Standard agronomic practices were followed for evaluating the genotypes during crop season. Randomly selected five plants from each replication were utilized for measurement of yield attributes *viz*., plant height, height of the first pod, number of pods per plant and seed yield of each genotype. Mature dry seeds from each genotype are randomly selected from harvested seeds from each replication and were used for estimation of protein and micro nutrients.

Protein content was measured by estimation of nitrogen in seed samples using sulfuric acid - selenium digestion. Total nitrogen content from aliquots of digests was measured by distillation with sodium hydroxide using Kjeldahl method (Kjeldahl, 1883). Total nitrogen content in powdered seeds was multiplied with a factor 6.25 to arrive at crude protein content (%) of grains (Jones, 1941). The micronutrients in the chickpea seeds (iron, zinc, copper and manganese) were analyzed by Atomic Absorption Spectroscopy (AAS) by measuring absorbance of the species at its resonance wavelengths. One gram of oven dried powdered seeds was digested with 10 ml of tri-acid mixture (HNO3: H2-SO4: HCLO4 @ 9:4:1). The volume of digested samples was made up to 100 ml. The filtered extract was used to measure the concentration of various elements by relative method using analytical grade solutions of elements of interest (Tandon, 1993).

Data were subjected to statistical analyses. The genotypic and phenotypic variances and coefficients of variation were calculated as per the formulae proposed by Burton (1952). Heritability in broad sense and genetic advance were calculated by the formulae given by Lush (1940) and Johnson *et al*. (1955) respectively. Genetic variability in genotypes was measured in terms of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) and were classified as low (<10 per cent), moderate (10-20 per cent) and high (>20 per cent) as per Siva Subramanian and Madhava Menon (1973). Genetic diversity study among the chickpea genotypes was arrived as per Mahalanobis (1936) and Rao (1952).

RESULTS AND DISCUSSION

The replication wise data collected from the genotypes were subjected to analysis of variance (Table 1) which revealed significant variances among the twenty four genotypes for protein, iron, zinc, copper and manganese contents and yield attributing traits *viz*., plant height, height of the first pod, number of pods per plant and seed yield. This clearly indicated that genotypes used in the current study possessed variable levels of attributes measured.

Table 1. Analysis of variance for protein content, micronutrients and yield attributes in *desi* chickpea

S. No.	Character	Mean sum of squares		
		Replications	Treatments	Error
1	Protein (%)	0.083	36.79**	3.38
2	Iron (mg/100 g)	2.240	17.07**	2.85
3	Zinc (mg/100 g)	0.061	18.44**	0.16
4	Copper (mg/100 g)	0.004	0.065**	0.011
5	Manganese (mg/100 g)	0.228	0.67**	0.26
6	Plant height	3.151	39.04**	4.22
7	First pod height	8.926	46.42**	1.24
8	Number of pods/ plant	121.92	489.55**	83.32
9	Seed yield per plant	37.45	59.55**	13.43

* Significant at P d≤ 0.05, ** Significant at P d≤ 0.01

Per se performance of genotypes for protein content and micro nutrients

Assessment of genetic variability in available germplasm or breeding lines for nutrients and protein content is one of the competent methods to identify potential genotypes for breeding strategy (Dwivedi *et al*., 2012). The performance of the genotypes with respect to protein, micro nutrients and yield attributes was presented in Table 2. Among the genotypes studied, ICC 14815 showed the highest protein content (32.7 %). Ten more genotypes in the present study also recorded significantly higher protein content than the general mean (21.1 %) whereas ICC 14831 (16.0 %), ICC-1398 (16.1 %) showed the lowest protein content. Sharma *et al.* (2013) and Jadhav *et al.*(2015) reported wide variability in protein content of chickpea genotypes.

Around the globe, more than two billion people are thought to be affected by an often invisible form of malnutrition due to micronutrient deficiencies in the diet leading to hidden hunger (Hodge, 2016). In this context, minerals are of great importance as they help in body growth, development, and to stay healthy and should be taken up through food. More than one third of the world's population is affected by iron and zinc deficiencies, which are ranked fifth and sixth among the ten most important risk causes of illness and disease in low income countries (WHO, 2002). Although several efforts have been made to estimate the genetic variability of chickpea genotypes for micro nutrient content, still detailed studies are required to understand the adaptable range of different nutrients for genetic bio-fortification programs. In the present study, for iron, genotypes ICC 14831 (18.5 mg/100g)

Table 2. *Per se* performance of 24 chickpea genotypes for protein content, micronutrients and yield attributes.

S. No.	Particulars	Protein (%)	Iron (mg/ 100 g)	Zinc (mg/ 100 g)	Copper (mg/ 100 g)	Manganese (mg/ 100 g)	Plant height (cm)	Height of the first pod (cm)	Number of pods/ plant	Seed yield (g/ plant)
1	ICC-16524	23.2	13.6	5.13	0.79	2.74	51.0	26.7	56.2	13.00
2	ICC-1397	17.6	8.4	4.34	0.59	2.85	40.5	18.2	55.2	7.30
3	ICC-1398	16.1	7.2	7.11	0.82	2.85	50.2	31.7	60.5	14.20
4	ICC-13524	19.4	6.8	5.06	0.70	4.30	44.0	21.9	57.4	14.55
5	ICC-1194	22.6	10.2	4.63	0.72	2.79	45.7	30.4	27.0	14.15
6	ICC-9942	23.0	7.7	4.98	0.77	2.79	38.5	18.0	45.5	19.00
7	ICC-10939	25.7	7.0	4.02	0.31	3.07	50.4	29.7	44.4	17.40
8	ICC-14402	32.6	12.3	5.12	0.61	3.15	44.1	24.5	82.0	5.45
9	ICC-14815	32.7	9.2	4.34	0.81	3.15	42.0	23.4	33.0	4.80
10	ICC-14831	16.0	18.5	5.22	0.61	2.78	38.9	24.4	39.4	13.20
11	ICC-5383	19.8	14.7	4.85	0.62	2.62	48.9	27.0	65.9	14.05
12	ICC-5434	17.2	8.8	3.78	0.61	3.29	50.4	22.5	71.9	7.50
13	ICC-10945	19.0	8.6	4.74	0.89	3.22	53.3	28.4	30.5	21.20
14	ICC-5845	19.1	8.3	2.64	0.79	3.96	49.3	26.3	42.7	18.90
15	ICC-8621	19.3	8.7	3.27	0.54	3.91	46.0	13.5	36.0	20.30
16	ICC-867	21.7	11.6	5.02	0.55	3.94	48.3	24.5	55.2	3.70
17	ICC-8740	21.0	6.1	4.08	0.83	2.27	47.0	22.7	32.5	7.05
18	ICC-6874	21.4	9.9	3.72	0.95	4.37	50.2	31.2	33.5	7.10
19	ICC-2942	17.2	8.6	4.21	0.86	3.71	50.5	27.9	63.5	13.75
20	ICC-7441	18.4	10.2	4.58	1.01	3.22	41.4	23.4	25.5	6.10
21	ICC-67	19.1	10.3	5.68	0.78	2.39	50.4	27.2	29.0	4.90
22	ICC-14194	22.3	12.3	3.61	0.89	3.39	39.5	17.0	63.9	10.50
23	ICC-1205	21.4	13.4	3.28	1.19	3.30	49.9	31.7	36.4	7.40
24	ICC-14669	22.0	12.1	3.12	0.80	3.84	45.9	27.0	46.3	6.80
Grand Mean		21.1	10.2	4.44	0.75	3.24	46.5	24.9	47.2	11.35
SEm±		1.30	1.20	0.28	0.07	0.37	1.45	0.79	6.45	2.59
CD at P≤ 0.05		3.80	3.50	0.83	0.22	1.07	4.25	2.30	18.9	7.58
CD at P≤ 0.01		5.16	4.75	1.13	0.29	1.45	5.77	3.13	25.6	10.29
CV %		8.7	16.6	9.1	14.0	16.0	4.4	4.5	19.3	32.1

followed by ICC 5383 (14.7 mg /100g) recorded significantly higher contents (Table 2). With respect to zinc, ICC 1398 (7.11 mg/100g) and ICC 67 (5.68 mg/ 100) recorded significantly higher content amongst the genotypes tested. For copper, two genotypes, ICC 1205 (1.19 mg/100g) and ICC 7441 (1.01 mg/100g) recorded significantly higher content against other varieties. ICC 6874 (4.37 mg/100g) and ICC 13524 (4.30 mg/100g) recorded the highest and significant manganese content. These potential genotypes can be exploited in breeding programs for developing micronutrient loaded chickpea cultivars after rigorous evaluation for their combing ability.

Per se performance of genotypes for metric traits

Plant height and height of the first pod from ground level have relevance in breeding for chickpea genotypes suitable for mechanical harvest which is one of the priority areas of research in chickpea breeding. Two genotypes *viz*., ICC 10945 (53.3 cm) and ICC 16524 (51.0 cm) recorded significantly higher mean values for plant height. ICC 1398 (31.7 cm), ICC 1205 (31.7 cm), ICC 6874 (31.2 cm), ICC 1194 (30.4 cm), ICC 10939 (29.7 cm) and ICC 2942 (27.9 cm) recorded significantly higher mean values for height of the first pod. Superior genotypes for number of pods were ICC 14402 (82.0) and ICC 5434 (71.9) whereas ICC 10945 (21.2 g/plant), ICC 8621 (20.3 g/plant) and ICC 9942 (19.0 g/plant) recorded significantly higher mean values for seed yield (Table 2).

Genetic variability for protein content, micro nutrients and yield attributes

The genetic gain expected through phenotypic selection in breeding programs can be predicted by considering genotypic coefficient of variation along with heritability estimates. Genetic parameters of variability *viz*., GCV, PCV, heritability and genetic advance (GA) as per cent mean were calculated and presented in table 3. Higher GCV and PCV were observed for iron, zinc, copper, number of pods and seed yield per plant. Moderate values were recorded for protein, manganese and height of the first pod and low values were recorded for plant height. The relative

Table 3. Genetic variability for protein content, micronutrients and yield attributes in *desi* chickpea

S. No	Character	Mean	Range	GCV (%)	PCV (%)	Heritability (%)	GA as % of mean
1	Protein (%)	21.1	16.0 – 32.7	19.34	21.21	0.83	36.3
2	Iron (mg/100 g)	10.2	6.1 – 18.5	26.20	31.02	0.71	45.6
3	Zinc (mg/100 g)	4.44	2.64 – 7.11	20.68	22.58	0.84	39.0
4	Copper (mg/100 g)	0.75	0.31 – 1.19	22.07	26.15	0.71	38.4
5	Manganese (mg/100 g)	3.24	2.27 – 4.37	13.85	21.14	0.43	18.7
6	Plant height (cm)	46.5	38.5 – 53.3	8.97	10.00	0.80	16.5
7	Height of the first pod (cm)	24.9	13.5 – 31.7	19.06	19.57	0.95	38.2
8	Number of pods per plant	47.2	25.5 – 82.0	30.19	35.85	0.71	52.4
9	Seed yield per plant (g)	11.35	3.7 – 21.2	42.32	53.24	0.63	69.3

Table 4. Clustering pattern in *desi* chickpea genotypes

Cluster Number	No of genotypes	Genotype(s)
I	6	ICC 67,ICC 8740,ICC 13524,ICC 16524, ICC 10939, ICC 8621
II	**9**	ICC 2942,ICC 9942,ICC 6874,ICC 14831, ICC 14815,ICC 5434,ICC 7441,ICC 1205, ICC 5845
III	4	ICC 10945, ICC 867,ICC 1397,ICC 5383
IV	**1**	ICC 1398
V	**1**	ICC 14669
VI	**2**	ICC 14194, ICC 14402
VII	**1**	ICC 1194

amount of heritable portion for different characters was assessed by estimating broad sense heritability. Higher heritability (above 60%) was recorded for protein, micronutrients and other yield traits studied except for manganese where medium heritability was recorded. High heritability coupled with high genetic advance was noticed for protein, iron, zinc, copper contents and also yield traits except plant height. This could be due to additive gene action and phenotypic selection could be effectively be exerted on these traits for improvement. High heritability with moderate GA was observed for plant height and low heritability with low GA for manganese content revealed that additive and non-additive gene action could be governing the trait. Slow progress is expected through selection for these traits.

Jayalakshmi *et al.* (2019) studied genetic variability for protein and micro nutrients in advance breeding lines and chickpea varieties grown in Andhra Pradesh and reported moderate to high genotypic variability for protein and micro nutrient content with high heritability and genetic advance. In a study with 27 chickpea cultivars grown in India, Jayalakshmi and Trivikrama Reddy (2018) reported higher heritability with high genetic advance for copper, zinc and manganese contents.

Genetic diversity studies

Plant genetic diversity offers opportunity for researchers to develop new improved varieties with desirable traits, which accommodates both farmers and breeders preferred traits. In the crossing of different genetic materials, the superior performance and desirable segregants are expected as compared to the crossing of similar genetic materials. Mahalanobis D^2 statistics manifests very successful method that is relied on the genetic variability analysis which is a good indicator of genetic diversity (Begna, 2021). Genetic diversity assessment in 24 chickpea genotypes of the present study categorized into seven distinct clusters (Tables 4 and 5). Nine genotypes were grouped into cluster II followed by six genotypes in cluster I, four genotypes in cluster III, two genotypes in cluster VI and cluster IV, V and VII had only one genotype each. Among the yield traits studied, 41.67 % genetic diversity was contributed by are height of the first pod followed by number of pods per plant (24.64%) (Table 6). Genetic diversity due to seed yield and plant height was 7.61 and 3.99 per cent, respectively. Among nutritive traits, protein content contributed upto 7.97 per cent followed by zinc (5.8%) copper (3.62%) and iron (2.17%).

Table 5. Genetic diversity as expressed by average inter and intra cluster distances

Cluster	I	II	III	IV	V	VI	VII
I	26.53	60.40	176.18	40.10	89.03	82.34	62.25
II		34.01	74.08	55.81	51.08	64.86	114.11
III			19.46	160.26	75.51	105.04	248.52
IV				0.00	86.88	55.73	78.21
V					0.00	74.41	117.23
VI						26.64	107.36
VII							0.00

Table 6. Relative contribution of protein content, micronutrients and yield attributes for genetic diversity.

Character	Times ranked First	Contribution (%)
Protein content	22	7.97
Iron conten	6	2.17
Zinc conten	16	5.8
Copper conten	10	3.62
Manganese conten	7	2.54
Plant height	11	3.99
Height of the first pod	115	41.67
Number of pods/ plant	68	24.64
Seed yield / plant	21	7.61

Based on *per se* performance and genetic diversity of the genotypes, promising genotypes should be chosen for future breeding programmes. Promising genotypes for protein and micro nutrients were diverse and grouped into different clusters. ICC 14402 with higher protein content and more number of pods per plant was grouped in cluster VI. ICC 1398 in Cluster IV with higher zinc content and height of the first pod while ICC 9942 for high seed yield per plant, ICC 6874 for high manganese content, ICC 14831 for high iron and ICC 7441 for higher content of copper was in cluster II. Jayalakshmi *et al*. (2019) reported five different clusters in advance breeding lines and chickpea varieties grown in Andhra Pradesh and that high protein lines were grouped in cluster I and lines with higher contents of micro nutrients were grouped in clusters IV and V.

This study revealed substantial genetic variability among 24 *desi* chickpea genotypes for traits contributing to yield, machine harvestable traits and also protein content and micronutrients iron, zinc, copper and manganese. Promising genotypes of the present study *viz*., ICC 14402, ICC 1398, ICC 9942 , ICC 6874 , ICC 14831 and ICC 7441 which are assorted into special clusters hold promise in breeding nutritionally rich chickpeas *vis a vis* addressing breeding for high yield and also designing genotypes amenable for mechanical harvest.

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Screening of pea (*Pisum sativum*) lines and varieties against downy mildew caused by *Peronospora viciae*

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ABSTRACT

Pea is affected by a number of fungal, bacterial, nematode and viral diseases and amongst these, downy mildew caused by *Peronospora viciae*, is considered most important, especially under cool and humid conditions. As the use of host plant resistance is considered the best way to manage downy mildew, an experiment was carried out to identify a good resistant source from forty five pea lines and varieties by screening against downy mildew pathogen. The germplasm Pant P244 showed resistant reaction while germplasm P222 showed moderately resistant reaction; of remaining, forty were moderately susceptible and 3 were found susceptible. Pant P 244 and P 222 showed low AUDPC value (103.3 and 113.8) with slow infection rate as compares to other susceptible genotypes that had AUDPC value between 411.13 -525.0. Thus, Pant P 244 and P 222 can be cultivated with reduced number of fungicidal spray to obtain maximum yield and minimal downy mildew severity.

Key words: AUDPC, Germplasm, Infection rate, Resistant, Susceptible

INTRODUCTION

India is the largest producer, consumer and importer of pulses in the world. It occupies an area of around 130.76 lakh hectares under pulses with annual production of 80.46 thousand quintals (Anonymous, 2020). Pulses are one of the most important sources of energy and basic protein that ranges from 18 to 25 per cent, making them one of the cheapest sources of protein available for human consumption. They are also rich in minerals like iron, magnesium, zinc and potassium and vitamins (Anonymous, 2017b). Yet, pulses face a number of biotic (bacterial, fungal and viral diseases) challenges that affects their productivity and production. They have low production due to less improved varieties. Major diseases include blight, *Fusarium* wilt, root rot in chickpea; , Green and Black gram leaf crinkle virus, yellow mosaic disease, mungbean phyllody disease, *Cercospora* leaf spot, powdery mildew in mungbean and urdbean;, lentil *Ascochyta* Blight, *Stemphylium* Blight, anthracnose, botrytis grey mold, lentil rust in lentil; and soya bean charcoal rot, white mold, Louisiana, root rot, black root rot in soybean. These diseases can be controlled by many physical as well as biological methods that have low cost of production and are also safe for environment (Iqbal *et al*., 2019).

Higher production and larger imports has led to marginal increase in pulse consumption estimated at around 50 grams per day in 2014-15 in comparison to 40 grams prior to 2012-13. And this level of consumption is being maintained in 2015-16 (Anonymous, 2017a). Among all pulses, larger imports of dry peas in recent years due its lower international prices have resulted in its increased share in the domestic pulse consumption. Because of the biotic and abiotic stresses there is high level of fluctuations in pulse production and prices therefore; farmers are not very interested on taking up pulse as cultivation. Low yields in India are because of poor spread of improved varieties and technologies, climatic changes, vulnerability to pests and diseases and generally declining growth rate of total factor productivity. Pea is affected by a number of diseases caused by fungi (rust, powdery mildew, downy mildew, root rot, *Alternaria* blight, *Aschochyta* blight, wilt, anthracnose, *Cercospora* leaf spot, damping off, seedling rot etc.), bacteria (bacterial blight and brown spot), nematodes (cyst nematode, lesion nematode and root-knot nematode) and viruses (cucumber mosaic virus, pea early browning virus, pea enation mosaic, pea mosaic, pea seed borne mosaic, pea streak and pea stunt). These diseases, under favourable conditions, may

cause significantly decrease in both yield and quality (Anonymous, 2021).

Amongst these, the downy mildew of pea caused by *Peronospora viciae* is a major constraint for pea production (Singh *et al*, 2020). Downy mildew is a limiting factor for pea production and is widespread in many other countries, including Sweden, New Zealand and India (Falloon *et al*., 2000; Stegmark, 1994; Thakur and Mathur, 2002). The disease has been reported to cause substantial yield loss leading to reduction in pod numbers by 65% and seed yield by 75% (Chang *et al*., 2013). The disease can be controlled by applying several seed treatment fungicides *viz*. metalaxyl-based products have produced the highest yield. Several foliar-applied fungicides, including pyraclostrobin, azoxystrobin and metalaxyl, have reduced downy mildew severity (Chang *et al*., 2013). The use of host plant resistance is considered the best means of downy mildew management in pea (Bayaa et al. 1998). Screening of pea germplasm under field conditions for resistance to downy mildew has been reported from India and continuous efforts were made from few decades to find a good source of resistance in pea against downy mildew disease. Currently, there is no single variety showing complete resistance against downy mildew pathogen. Therefore, the present experiment was carried out in search for good resistance against pea downy mildew.

MATERIAL AND METHODS

Based on the severity of downy mildew disease, Pantnagar has been designated as one of the AICRP (MULLaRP) centre for screening of pea germplasm against downy mildew disease. A total of forty five pea lines/varietes obtained from AICRP (MULLaRP) were used in this study. The germplasm was screened under natural epiphytotic condition for over two seasons (*Rabi* 2013-2014 and 2014-2015). The lines/ varietal screening was undertaken following 'Infector row technique'. Each entry was sown in 2 m row length at 30 cm row spacing with a susceptible check Arkel after every test entries. The disease incidence and severity was recorded when first symptoms appear and subsequent observations were recorded at seven days interval.

$$\text{Percent Disease Severity (\%)} = \frac{\text{Percent of leaf area showing symptoms}}{\text{Total leaf area in row}} \times 100$$

Each plant was rated for downy mildew severity on Davidson's modified (Davidson et al. 2004) 0-5 scale:

To compare the different genotypes for their resistance 'A' value and 'r' value were calculated for each genotype. Correlation coefficients of AUDPC with pustule size were also estimated using Karl Pearson's correlation coefficient (r).The following parameters was calculated during the study.

Table 1. Disease severity scale with disease reaction.

Rating	Description	Reaction
0	No symptoms on leaf	Immune (I)
1	Sporulation on 5% of the leaf and stem	Resistant (R)
2	Sporulation on 5.1–10% of leaf and stem	Moderately resistant (MR)
3	Sporulation on 10.1–30% of the leaf and stem	Moderately susceptible (MS)
4	Sporulation on 30.1-60% of leaf and stem	Susceptible (S)
5	Sporulation on greater than 60% of leaf and stem	Highly susceptible (HS)

AUDPC (A) value

Downy mildew incidence was quantified using the following formulae (Nagarajan and Muralidharan, 1995).

$$A = \sum_{i=1}^{k} \frac{1}{2}(S_i + S_{i-1})d$$

Where S_i = Disease incidence at the end of the week i, k = Number of successive evaluations of disease, and d = Interval between two evaluations.

Apparent rate of infection ('r')

The apparent rate of infection was calculated using Vanderplank (1968) formula:

$$r = \frac{2.303}{t_2 - t_1} \log \frac{x_2(1 - x_1)}{x_1(1 - x_2)}$$

where, r is the apparent infection rate in non-logarithmic phase, x_1 is the disease index at initial week time (t_1), x_2 is the disease index at subsequent week time (t_2)

RESULTS AND DISCUSSION

Host plant resistance is considered the most adequate among all management strategies to control downy mildew. Screening of field pea germplasms under field conditions for resistance to downy mildew has been reported in India but only moderate levels of resistance are available in commercial cultivars. Severity of downy mildew is greatly influenced by the environment during infection initiation and disease development. With this in mind, present investigation was carried out to screen lines/varieties for resistance through phenotypic screening approach. Symptoms that appeared in downy mildew of pea have been

Table 2: Screening of germplasm against downy mildew 2013-14 & 2014-15 (pooled).

Sl. No.	Germplasm	% Disease severity			'A' Value*	'r' Value*	Disease Rating scale	Reaction group	Yield (kg/ha)	Test weight (g)
		2013	2014	Mean						
1	Pant P 244	4	5	4.5	103.3	0.1904	1	R	1950.5	129
2	Pant P 42	25	20	22.5	306.3	0.2026	3	MS	2012.75	134
3	KPF 12-04	15	15	15	169.8	0.2122	3	MS	1743	133
4	KPMR 522 (VC)	30	25	27.5	402.5	0.2026	3	MS	1535.5	141.5
5	HFP 4 (VC & SC)	20	15	17.5	250.3	0.1904	3	MS	1867.5	129
6	HFP 530	20	20	20	269.5	0.1973	3	MS	2158	145.1
7	HFP 1016	30	25	27.5	358.8	0.2122	3	MS	2054.25	131.4
8	HFP 9907(VC)	20	15	17.5	250.3	0.1904	3	MS	1805.25	130
9	HFP 8909 (VC)	15	12	13.5	178.5	0.1947	3	MS	2054.25	142.15
10	KPMR 925	25	20	22.5	306.3	0.2026	3	MS	1328	127.5
11	Pant P 200	20	15	17.5	250.3	0.1904	3	MS	1867.5	137.75
12	VL 202	15	10	12.5	161.0	0.1904	3	MS	892.25	92.45
13	Pant P 233	25	20	22.5	306.3	0.2026	3	MS	1514.75	78
14	VL 59	20	12	16	204.8	0.2016	3	MS	3735	67.7
15	Pant P 222	10	10	10	113.8	0.1929	2	MR	477.25	68
16	Pant P 217	25	20	22.5	306.3	0.2026	3	MS	2801.25	53.5
17	Pant P 213	30	20	25	269.5	0.1973	3	MS	1307.25	78.1
18	Pant P 243	20	15	17.5	236.3	0.2137	3	MS	1888.25	59.5
19	VL 58	30	30	30	428.8	0.2069	3	MS	3237	59
20	RPG 79	35	35	35	507.5	0.2074	4	S	1826	58
21	NDP 12-102	25	25	25	318.5	0.2132	3	MS	456.5	50.7
22	KPMR 853	30	30	30	428.8	0.2069	3	MS	269.75	57.6
23	Pant P 195	25	25	25	332.5	0.2079	3	MS	565.645	70
24	HUDP 1302	20	25	22.5	274.8	0.2408	3	MS	3320	61
25	RFP 2009 2-1	40	35	35	525.0	0.2244	4	S	1307.25	65
26	HUDP 1209	20	20	20	225.8	0.2137	3	MS	477.25	54.15
27	RFP 2009 -2	40	25	32.5	498.8	0.2037	3	MS	859.05	72.5
28	RFP 2009 -3	35	35	35	411.3	0.2074	4	S	435.75	60.4
29	HUDP 1301	15	20	17.5	246.8	0.2069	3	MS	1224.25	65.5
30	KPMR 851	20	25	22.5	280.0	0.2190	3	MS	439.9	61.65
31	KPM 928	15	25	20	232.8	0.2244	3	MS	402.55	65
32	HUDP 15 (VC)	10	15	12.5	120.8	0.2122	3	MS	477.25	52.5
33	IPFD 13-14	15	15	15	176.8	0.2001	3	MS	439.9	131
34	IPFD 99-2Z	25	25	25	315.0	0.2079	3	MS	585.15	131
35	IPFD 13-2	30	30	30	428.8	0.2069	3	MS	518.75	140
36	IPF 10	25	25	25	306.3	0.2074	3	MS	601.75	146
37	IPFD 13-2	10	15	12.5	129.5	0.2026	3	MS	1162	151
38	IPF 2-17	25	25	25	329.0	0.2132	3	MS	1950.5	144.1
39	IPFD 11-5	25	20	22.5	309.8	0.1982	3	MS	680.6	139.5
40	IPF 5-19	15	15	15	187.3	0.2001	3	MS	3008.75	163
41	IPFD 99-13	20	15	17.5	252.0	0.2069	3	MS	726.25	145
42	IPFD 11-5	25	25	25	315.0	0.2079	3	MS	2365.5	158.8
43	IPFD 12-2	15	20	17.5	203.0	0.2069	3	MS	505.926	131.5
44	IPFD 13-3	20	25	22.5	280.0	0.2190	3	MS	2178.75	140.5
45	IPFD 13-4	20	20	20	257.3	0.2057	3	MS	975.25	139.5

Area under disease progress curve (AUDPC), 'r' – Apparent rate of infection, MR-moderately resistant, MS-moderately susceptible, S- susceptible, HS-highly susceptible

presented in Fig 1 along with the microscopic study in Fig 2.

Year 2013-2014

A total of forty five lines/varieties were screened under epiphytotic field conditions over two seasons. Among different genotypes during 2013-2014, disease severity was as high as 40 % in RPG 79, RFP 2009 2-1, RFP 2009 2 and RFP 2009-3 and as low as 4 % in Pant P244. Maximum AUDPC value was observed in RPG 79, RFP 2009 2-1, RFP 2009 2 and RFP 2009-3 (577.5) and least AUDPC (80.5) was observed in Pant P 244.

Fig. 1. Symptoms of downy mildew. The disease is characterized by the development of a mass of greyish mycelium that mostly covers the lower surface of foliage and corresponding upper surface shows chlorotic zone

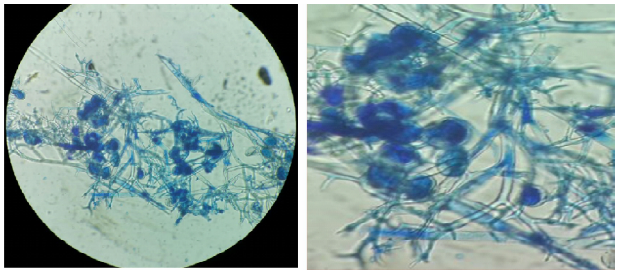

Fig. 2. Microscopic view showing dichotomous branching of hypha along with oval conidia

Apparent rate of infection was maximum in HUDP 1302 (0.1095) whereas P 42 showed lowest infection rate (0.022**)** (Table 2).

Year 2014-2015

Of the different lines/varieties screened during 2014-2015, disease severity was of a high of 35 per cent in RPG 79, RFP 2009 2-1 and RFP 2009-3 and as low as 5 per cent in Pant P 244. Maximum AUDPC value was observed in RFP 2009 2-1 (472.5) and least AUDPC (108.5) was observed in HUDP 15. Apparent rate of infection was maximum in RPG 79 (0.3882) whereas P 244 showed lowest infection rate (0.3286) (Table 2).

Pooled data of 2013-2014 to 2014-2015

During both the years, among the 45 total genotypes, the per cent disease severity was maximum in RPG 79, RFP 2009 2-1 and RFP 2009-3 (35) and minimum in Pant P 244 (4.5). Maximum AUDPC value was observed in RFP 2009 2-1 (525.0) while Pant P 244 scored least AUDPC (103.3). Apparent rate of infection was maximum in RFP 2009 2-1 (0.2244) whereas Pant P 244 showed lowest infection rate (0.1904. (Table 2).

Among genotypes screened none was free from downy mildew during both seasons. Only one genotype revealed a resistant reaction with 5 % disease severity (Pant P 244). Maximum numbers of genotypes fall under the category of moderately susceptible (40) followed by susceptible (3.), resistant (1.) and moderately resistant (1). Genotypes *viz.*, RPG 79, RFP 2009 2-1and and RFP 2009 3 fell under the category of susceptible with more than 35 % incidence. Similar works has been done by Bhushan et al., 2013 where he found 'P-89' germplasm to be the most suitable genotype with minimum downy mildew incidence and highest under mid hill conditions. Likewise, Stegmark (1990) found moderately resistant reaction in old cultivar 'Dark Skin Perfection' against downy mildew infestation of leaves and pods. Similarly, Kumar et al. (1994) used area under disease progress curve (AUDPC) to depict the overall disease stress that the plants were subjected to and described the pea varieties Pant P8, HUP 8063, KPMR 22 to possess good level of partial resistance. Buckseth and Singh, 2017 have done similar screening works in pea against powdery mildew and rust pathogen but no genotype was found resistant. Upadhyay et al, 2017 **a**lso have done screening of pea germplasms against rust. They have found that Pant P 244 and Pant P 42 showed moderate resistant with low AUDPC value and slow infection rate. They have also observed that pustules appeared on moderate resistant genotypes were small (1.5-1.7mm) as compare to susceptible genotypes.

CONCLUSION

It can thus be concluded that the two genotypes, namely Pant P 244 and Pant P 222, showed resistant reaction and moderately resistant reaction respectively against downy mildew and can be a good option to integrate it with reduced number of fungicidal sprays to obtain maximum yield and minimal downy mildew severity.

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Yield loss and management of downy mildew on field pea in Alberta, Canada

Correlation and path coefficient analysis for yield and its contributing characters in advanced breeding lines of field pea (*Pisum sativum* L.)

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ABSTRACT

In the present investigation, 47 advanced breeding lines of field pea were utilized to study the correlation and path analysis for yield and its contributing characters. The experiment was conducted in Randomized complete block design with three replications during *Rabi*, 2020-2021 at Andro Research Farm, CAU, Imphal, Manipur. Ten plants were selected at random from each breeding line in each replication for recording data on 13 quantitative characters. Analysis of variance revealed that highly significant differences among the lines for all the characters studied. Seed yield per plant had shown positive and significant correlation with number of seed per plant, biological yield per plant, plant height, number of pod per plant, number of seed per pod and harvest index at both genotypic and phenotypic level. The maximum positive direct effect on seed yield per plant was exhibited by biological yield per plant and harvest index followed by number of seed per plant at both genotypic and phenotypic level. Therefore, these characters can be considered for selecting lines to improve the seed yield in the field pea.

***Key words*:** Correlation, Field pea, Path analysis, Quantitative characters

INTRODUCTION

Pea (*Pisum sativum* L.) is an annual self-pollinated, temperate legume, belongs to family Leguminosae (Fabaceae) having diploid chromosome number, 2n=2x=14. It is of Mediterranean origin; the Near East and Ethiopia are considered as its secondary centers of origin (Blixt 1974). Field pea is an important rabi pulse crop which is highly productive and is grown not only for food but also for animal feed. It is the second most important food legume worldwide after *Phaseolus vulgaris* L. (Tar'an *et al.* 2005). It is grown in an area of about 0.61 million hectares in India with production 0.81 million tons and productivity of 1,337.8 kg/ha (Anonymous 2019). In India, it is grown in Uttar Pradesh, Madhya Pradesh, Jharkhand, Assam, Maharashtra and Bihar. The measurement which estimates the connection between factors is called as correlation coefficient. It quantifies the connection between various plant traits and decides the constituent parameters by them yield could be improved through selection. Yield of the crop can be improved through indirect selection for highly heritable traits, which are related with the yield (Singh 1983). Path analysis is an imperative means for division of correlation coefficients into direct and indirect effects of independent variables on dependent variable. It has been generally used to recognize attributes that have vast impact on yield and related traits for potential use in selection programme. Singh *et al*. (2007) found that the biological yield per plant, harvest index, pods per plant, number of branches per plant and grains per pod were significantly correlated with seed yield per plant. Singh *et al*. (2011) reported that number of pod per plant, plant height, number of primary branches per plant, 100 seed weight and number of cluster per plant were the major characters contributing to grain yield as these traits were significantly and positively associated with grain yield per plant. Jeberson *et al*. (2016) revealed that positive and highly significant association of yield with plant height, cluster/plant, pods/plant, pod length, seeds/pod and 100 seed weight. Bhardwaj *et al*. (2020) reported that total biomass and harvest index had maximum positive direct effects on seed yield/plant. Therefore, the present investigation was carried out with the objective to estimate correlation and path coefficient analysis for yield and its contributing characters.

MATERIALS AND METHODS

The experiment was conducted on 47 field pea advanced breeding lines (Table 1) at Andro Research

Farm, CAU, Imphal, Manipur during *Rabi* 2020-21, following Randomized Complete Block Design with three replications. The research farm is located at 24^0 46´ N and 94^0 03´ E. Each breeding line was sown in a single row of 4m length with a spacing of 30 cm × 10 cm between and within the rows. Ten plants were selected at random from each line in each replication for recording data.

Table 1. List of Field pea (*Pisum sativum* L.) advanced breeding lines (in yield) and their source of origin

SI. No.	Genotypes	Sources
1.	CAU-FP-1	AICRP(MULLaRP) CAU, Imphal
2.	CAU-FP-2	AICRP(MULLaRP) CAU, Imphal
3.	CAU-FP-3	AICRP(MULLaRP) CAU, Imphal
4.	CAU-FP-4	AICRP(MULLaRP) CAU, Imphal
5.	CAU-FP-5	AICRP(MULLaRP) CAU, Imphal
6.	CAU-FP-6	AICRP(MULLaRP) CAU, Imphal
7.	CAU-FP-7	AICRP(MULLaRP) CAU, Imphal
8.	CAU-FP-8	AICRP(MULLaRP) CAU, Imphal
9.	CAU-FP-9	AICRP(MULLaRP) CAU, Imphal
10.	CAU-FP-10	AICRP(MULLaRP) CAU, Imphal
11.	FPT-20-16	IIPR, Kanpur
12.	FPT-20-17	IIPR, Kanpur
13.	FPT-20-18	IIPR, Kanpur
14.	FPT-20-19	IIPR, Kanpur
15.	FPT-20-20	IIPR, Kanpur
16.	FPT-20-21	IIPR, Kanpur
17.	FPT-20-22	IIPR, Kanpur
18.	FPT-20-23	IIPR, Kanpur
19.	FPT-20-24	IIPR, Kanpur
20.	FPT-20-25	IIPR, Kanpur
21.	FPT-20-26	IIPR, Kanpur
22.	FPT-20-27	IIPR, Kanpur
23.	FPT-20-28	IIPR, Kanpur
24.	FPT-20-29	IIPR, Kanpur
25.	FPT-20-30	IIPR, Kanpur
26.	FPT-20-31	IIPR, Kanpur
27.	FPT-20-32	IIPR, Kanpur
28.	FPT-20-33	IIPR, Kanpur
29.	FPT-20-34	IIPR, Kanpur
30.	FPT-20-35	IIPR, Kanpur
31.	HFP 1574	CCS HAU, Hisar
32.	IPF 19-11	IIPR, Kanpur
33.	IPF 19-15	IIPR, Kanpur
34.	IPF 19-18	IIPR, Kanpur
35.	KPMR 957	CSAUA&T, Kanpur
36.	Makhayatmubi	Landrace
37.	Pant P 473	Pantnagar
38.	Pant P 474	Pantnagar
39.	Pant P 476	Pantnagar
40.	RFPG 144	RARS, Durgapura
41.	RFPG 151	RARS, Durgapura
42.	RFPG 170	RARS, Durgapura
43.	RFP 2010-21	Raipur
44.	RFP 2012-122-1	Raipur
45.	VL 70	VPKAS, Almora
46.	VL 71	VPKAS, Almora
47.	WBFP-14-S-9	Berhampore (WB)

The different characters considered were days to 50% flowering, days to maturity, plant height (cm), number of primary branches per plant, number of cluster per plant, number of pod per plant, pod length (cm), number of seed per pod, number of seed per plant, 100 seed weight (g), seed yield per plant (g), biological yield per plant (g) and harvest index (%). The usual RBD analysis method was followed to determine the significant effects of treatments (Rangaswamy 2010). Phenotypic and genotypic correlations were worked out by using the formula suggested by Falconer (1967). The relative influence of twelve components traits on yield by themselves (direct effects) and through other traits (indirect effects) was evaluated by the method of path coefficient analysis as suggested by Dewey and Lu (1959).

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) for the design of the experiment under study was calculated separately for each trait. The mean sum of squares based on ANOVA of 47 field pea lines for 13 characters indicated the presence of high amount of variability among the lines. It was detected that estimated analysis of variance (Table 2) revealed that variance present in various lines for all the studied characters were highly significant.

Correlation coefficient analysis

In the present study, the values of genotypic correlation coefficients were slightly higher than their corresponding phenotypic correlation coefficients for all the characters, signifying that the association among various traits is of genetic cause rather than environmental effect (Table 3). Similar results were shown by Singh and Singh (2005), Dhama *et al.* (2010) and Jeberson *et al*. (2016) in pea.

Seed yield per plant had shown positive and significant correlation with number of seed per plant (0.83**), biological yield per plant (0.82**), plant height (0.53**), number of pod per plant (0.47**), number of seed per pod (0.45**), harvest index (0.43**), pod length (0.36*) and number of cluster per plant (0.30*) at genotypic level. At phenotypic level, seed yield per plant had shown positive and significant correlation with biological yield per plant (0.80**), number of seed per plant (0.79**), plant height (0.49**), harvest index (0.47**), number of pod per plant (0.44**), number of seed per pod (0.40**), pod length (0.31*) and number of cluster per plant (0.28*). These results are in

Table 2. Analysis of variance for 13 different characters in 47 breeding lines of field pea

Source of Variation	Degrees of Freedom	DF	DM	PH	PBR	CPP	PPP	PL
Replication	2	8.00	48.43**	15.67	0.02	0.11*	0.06	0.11
Genotypes	46	13.01**	10.03**	210.30**	0.25**	0.79**	3.12**	0.61**
Error	92	4.07	3.24	6.24	0.01	0.03	0.07	0.08
C.V. (%)		2.82	1.74	3.04	15.29	11.29	5.89	4.84

Source of Variation	Degrees of Freedom	SPP	SPPL	100 SW	SYP	BY	HI
Replication	2	0.05	1.34	4.01	0.02	0.09	5.70
Genotypes	46	1.29**	34.47**	14.84**	0.79**	5.16**	51.89**
Error	92	0.08	0.51	2.07	0.02	0.03	2.82
C.V. (%)		5.84	4.60	7.06	4.67	2.24	4.66

** 1% Level of significance * 5% Level of significance

DF- Days to 50% flowering, DM- Days to maturity, PH- Plant height, PBR- Number of primary branches per plant, CPP- Number of cluster per plant, PPP- Number of pod per plant, PL- Pod length, SPP- Number of seed per pod, SPPL- Number of seed per plant, 100 SW- 100 seed weight, BY- Biological yield per plant, SYP- Seed yield per plant, HI-Harvest index.

Table 3. Phenotypic correlation coefficient (upper diagonal) and genotypic correlation coefficient (lower diagonal) among yield and its contributing characters in 47 breeding lines of field pea

Characters	DF	DM	PH	PBR	CPP	PPP	PL	SPP	SPPL	100 SW	BY	HI	SYP
DF	**1.00**	0.36*	-0.13	-0.00	-0.16	-0.15	0.14	0.15	0.02	0.00	-0.02	0.05	0.02
DM	0.67**	**1.00**	0.05	-0.24	-0.08	-0.20	0.10	0.04	-0.04	0.00	0.11	-0.19	-0.01
PH	-0.30*	-0.05	**1.00**	-0.31*	0.09	0.21	0.26	0.28*	0.34*	0.28*	0.54**	-0.01	0.49**
PBR	-0.04	-0.42**	-0.34*	**1.00**	0.15	0.19	-0.26	-0.05	0.03	-0.35*	-0.17	-0.07	-0.20
CPP	-0.30*	-0.17	0.10	0.16	**1.00**	0.71**	-0.29*	-0.21	0.49**	-0.38**	0.11	0.28*	0.28*
PPP	-0.26	-0.38**	0.22	0.21	0.75**	**1.00**	-0.34*	-0.18	0.69**	-0.36*	0.32*	0.24	0.44**
PL	0.18	0.02	0.26	-0.30*	-0.38**	-0.45**	**1.00**	0.56**	0.05	0.30*	0.25	0.13	0.31*
SPP	0.24	0.08	0.30*	-0.04	-0.24	-0.20	0.65**	**1.00**	0.35*	0.01	0.33*	0.20	0.40**
SPPL	-0.02	-0.11	0.35*	0.03	0.52**	0.72**	0.03	0.37**	**1.00**	-0.32*	0.59**	0.40**	0.79**
100 SW	0.05	0.24	0.38**	-0.45**	-0.46**	-0.45**	0.49**	0.05	-0.37**	**1.00**	0.24	-0.05	0.18
BY	-0.00	0.16	0.57**	-0.17	0.11	0.34*	0.30*	0.36*	0.60**	0.30*	**1.00**	-0.15	0.80**
HI	0.06	-0.32*	-0.00	-0.09	0.31*	0.27	0.13	0.23	0.45**	-0.10	-0.15	**1.00**	0.47**
SYP	0.03	-0.03	0.53**	-0.22	0.30*	0.47**	0.36*	0.45**	0.83**	0.20	0.82**	0.43**	**1.00**

DF- Days to 50% flowering, DM- Days to maturity, PH- Plant height, PBR- Number of primary branches per plant, CPP-Number of cluster per plant, PPP- Number of pod per plant, PL- Pod length, SPP- Number of seed per pod, SPPL- Number of seed per plant, 100 SW- 100 seed weight, BY- Biological yield per plant, HI-Harvest index, SYP- Seed yield per plant.

**1% Level of significance *5% Level of significance

agreement with Tiwari and Lavanya (2012), Jeberson *et al*. (2016), Katoch *et al*. (2016), Gautam *et al*. (2017) and Kumar *et al*. (2018) in pea.

A positive correlation between desirable attributes is beneficial to the plant breeder, because it helps to bring the synchronized development in both the characters under study. Some of the characters which showed positive correlation with seed yield per plant and important for crop improvement are number of seed per plant, biological yield per plant, plant height, number of pod per plant, number of seed per pod, harvest index, pod length and number of cluster per plant.

Path coefficient analysis

The coefficient of correlation indicates only the relationship between two variables. However, it does not provide information regarding the extent of change in one variable resulting from the change in another variable. Seed yield per plant is an end product of various actions and interaction of different associated component characters. If a greater number of variables is interrelated, the association sometimes become in-variable. In such situation path coefficient analysis is very useful. Path coefficient analysis allows separating the cause and the effect, in other words, it allows the partitioning of correlation coefficient into direct and indirect causes and permits a critical examination of the specific forces acting to a given correlation and measures the relative importance of each causal factor (Wright 1921).

In the present investigation, the path analysis showing the direct and indirect effects of the different traits were shown in the table 4 and 5. Highest positive

Table 4. Direct (diagonal) and indirect effects of yield component traits on seed yield per plant at phenotypic level, in 47 field pea breeding lines

Characters	DF	DM	PH	PBR	CPP	PPP	PL	SPP	SPPL	100 SW	BY	HI
DF	**-0.0011**	-0.0004	0.0002	0.0000	0.0002	0.0002	-0.0002	-0.0002	0.0000	0.0000	0.0000	-0.0001
DM	-0.0005	**-0.0013**	-0.0001	0.0003	0.0001	0.0002	-0.0001	-0.0001	0.0001	0.0000	-0.0001	0.0002
PH	-0.0018	0.0006	**0.0139**	-0.0044	0.0013	0.0029	0.0037	0.0039	0.0047	0.0038	0.0075	-0.0002
PBR	0.0000	0.0025	0.0033	**-0.0104**	-0.0016	-0.0020	0.0028	0.0005	-0.0003	0.0036	0.0017	0.0008
CPP	-0.0026	-0.0013	0.0015	0.0025	**0.0165**	0.0116	-0.0049	-0.0035	0.0080	-0.0063	0.0018	0.0046
PPP	0.0045	0.0061	-0.0066	-0.0059	-0.0219	**-0.0310**	0.0105	0.0056	-0.0215	0.0112	-0.0099	-0.0076
PL	0.0040	0.0030	0.0078	-0.0080	-0.0088	-0.0100	**0.0296**	0.0166	0.0016	0.0090	0.0075	0.0037
SPP	-0.0047	-0.0012	-0.0085	0.0016	0.0065	0.0054	-0.0169	**-0.0301**	-0.0105	-0.0003	-0.0098	-0.0059
SPPL	0.0019	-0.0047	0.0398	0.0035	0.0573	0.0812	0.0062	0.0408	**0.1173**	-0.0379	0.0694	0.0475
100 SW	0.0000	0.0000	0.0074	-0.0093	-0.0102	-0.0097	0.0082	0.0003	-0.0086	**0.0268**	0.0064	-0.0013
BY	-0.0120	0.0895	0.4337	-0.1330	0.0877	0.2570	0.2031	0.2612	0.4746	0.1932	**0.8021**	-0.1189
HI	0.0298	-0.1037	-0.0070	-0.0400	0.1530	0.1335	0.0684	0.1071	0.2205	-0.0258	-0.0808	**0.5452**

DF- Days to 50% flowering, DM- Days to maturity, PH- Plant height, PBR- Number of primary branches per plant, CPP- Number of cluster per plant, PPP- Number of pod per plant, PL- Pod length, SPP- Number of seed per pod, SPPL- Number of seed per plant, 100 SW- 100 seed weight, BY- Biological yield per plant, HI-Harvest index.

direct effects on seed yield per plant exerted by biological yield per plant (0.8021) and harvest index (0.5452), followed by number of seed per plant (0.1173), pod length (0.0296), 100 seed weight (0.0268), number of cluster per plant (0.0165) and plant height (0.0139) at phenotypic level (Fig. 1). Highest positive direct effects on seed yield per plant exerted by biological yield per plant (0.7097) and harvest index (0.4900), followed by number of seed per plant (0.1776), pod length (0.1283), days to maturity (0.1118), number of pod per plant (0.0875), 100 seed weight (0.0532), number of primary branches per plant (0.0368) and plant height (0.0084) at genotypic level (Fig. 2). This

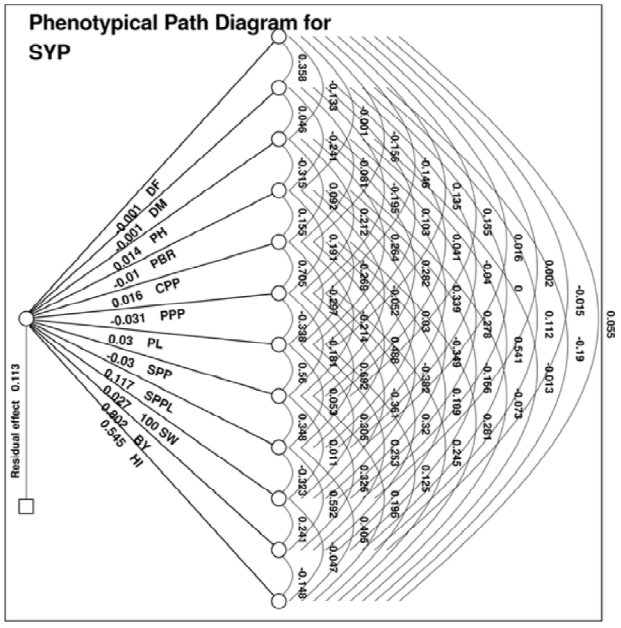

Fig. 1. Phenotypical path diagram for seed yield per plant

DF- Days to 50% flowering, DM- Days to maturity, PH- Plant height, PBR- Number of primary branches per plant, CPP- Number of cluster per plant, PPP- Number of pod per plant, PL- Pod length, SPP- Number of seed per pod, SPPL- Number of seed per plant, 100 SW- 100 seed weight, BY- Biological yield per plant, HI-Harvest index, SYP- Seed yield per plant

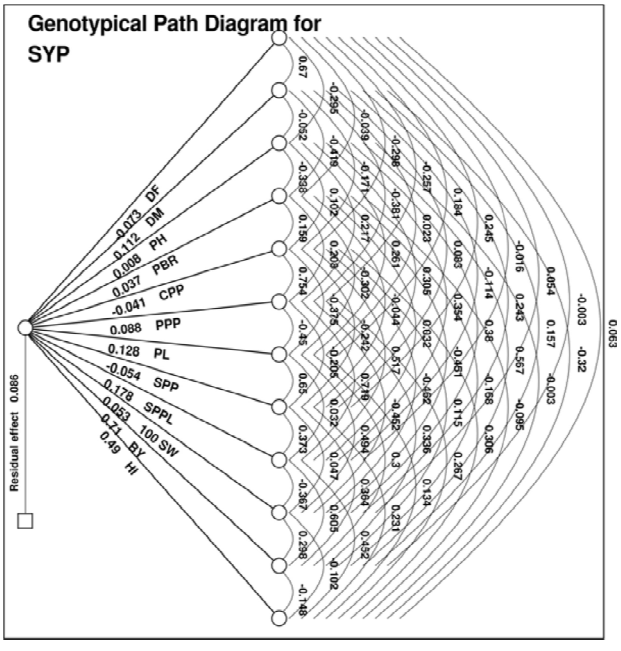

Fig. 2. Genotypical path diagram for seed yield per plant

DF- Days to 50% flowering, DM- Days to maturity, PH- Plant height, PBR- Number of primary branches per plant, CPP- Number of cluster per plant, PPP- Number of pod per plant, PL- Pod length, SPP- Number of seed per pod, SPPL- Number of seed per plant, 100 SW- 100 seed weight, BY- Biological yield per plant, HI-Harvest index, SYP- Seed yield per plant.

Table 5. Direct (diagonal) and indirect effects of yield component traits on seed yield per plant at genotypic level, in 47 field pea breeding lines

Characters	DF	DM	PH	PBR	CPP	PPP	PL	SPP	SPPL	100 SW	BY	HI
DF	**-0.0734**	-0.0492	0.0217	0.0029	0.0219	0.0189	-0.0135	-0.0180	0.0012	-0.0040	0.0002	-0.0046
DM	0.0749	**0.1118**	-0.0058	-0.0468	-0.0191	-0.0426	0.0025	0.0093	-0.0127	0.0272	0.0176	-0.0358
PH	-0.0025	-0.0004	**0.0084**	-0.0028	0.0009	0.0018	0.0022	0.0026	0.0030	0.0032	0.0048	0.0000
PBR	-0.0014	-0.0154	-0.0125	**0.0368**	0.0059	0.0077	-0.0111	-0.0016	0.0012	-0.0166	-0.0062	-0.0035
CPP	0.0123	0.0071	-0.0042	-0.0066	**-0.0413**	-0.0312	0.0155	0.0100	-0.0214	0.0191	-0.0047	-0.0127
PPP	-0.0225	-0.0334	0.0190	0.0182	0.0660	**0.0875**	-0.0394	-0.0179	0.0629	-0.0396	0.0294	0.0233
PL	0.0236	0.0029	0.0335	-0.0388	-0.0481	-0.0578	**0.1283**	0.0834	0.0041	0.0634	0.0385	0.0172
SPP	-0.0131	-0.0045	-0.0164	0.0024	0.0130	0.0110	-0.0349	**-0.0536**	-0.0200	-0.0025	-0.0195	-0.0124
SPPL	-0.0028	-0.0202	0.0628	0.0057	0.0918	0.1277	0.0057	0.0663	**0.1776**	-0.0652	0.1074	0.0802
100 SW	0.0029	0.0130	0.0202	-0.0240	-0.0246	-0.0241	0.0263	0.0025	-0.0196	**0.0532**	0.0159	-0.0054
BY	-0.0023	0.1115	0.4025	-0.1195	0.0815	0.2385	0.2130	0.2582	0.4293	0.2115	**0.7097**	-0.1048
HI	0.0309	-0.1568	-0.0014	-0.0464	0.1500	0.1306	0.0659	0.1132	0.2213	-0.0501	-0.0723	**0.4900**

DF- Days to 50% flowering, DM- Days to maturity, PH- Plant height, PBR- Number of primary branches per plant, CPP- Number of cluster per plant, PPP- Number of pod per plant, PL- Pod length, SPP- Number of seed per pod, SPPL- Number of seed per plant, 100 SW- 100 seed weight, BY- Biological yield per plant, HI-Harvest index.

result confirmed the importance of these characters for their contribution towards seed yield per plant. Lavanya *et al*. (2010) observed similar results that biological yield per plant and harvest index registered high positive direct effect on seed yield per plant at both genotypic and phenotypic levels in field pea. Lal *et al*. (2018) reported that highest positive and direct effect on seed yield per plant was exerted by harvest index in field pea. Bahadur and Devi (2021) also found that highest positive and direct effect on seed yield per plant was exerted by biological yield per plant and harvest index in field pea.

Further, in the present investigation, it is revealed that 89 per cent of the yield contributing characters were utilized in this data analysis as the residual effect was 0.11 (11%) (Fig. 1).

CONCLUSION

Of all the characters under study, number of seed per plant, biological yield per plant, plant height, number of pod per plant, number of seed per pod, harvest index, pod length and number of cluster per plant had highly significant and positive correlation with seed yield per plant and also had positive association among themselves. Hence, selection of these characters simultaneously would bring improvement in yield. The path analysis also indicated that the highest positive direct effect was shown by biological yield per plant, harvest index followed by number of seed per plant, pod length, 100 seed weight and plant height. Direct selection of these traits would be effective in improvement of seed yield per plant in field pea.

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An assessment of extension, technological gaps and income augmentation through participatory cluster front line demonstrations on chickpea (*Cicer arietinum*) in Rajasthan

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ABSTRACT

From 2015 to 2020, 13134 Cluster Front Line Demonstrations (CFLDs) on chickpea were conducted in 5417.45 ha area by Krishi Vigyan Kendras (KVKs) of the Rajasthan state under National Food Security Mission (NFSM). The data were solicited from partner farmers in the participatory approach. The study shows a higher yield of 17.94q/ha over farmers' practice (13.71q/ha). The technology gap of 5.75q/ha, extension gap of 4.23q/ha, and technology index of 24.27% observed. An average, additional yield of 4.23q/ha was observed in CFLDs. Consequently, Rs. 15337/ha has been added through CFLDs during the five years. In a nutshell, it constituted a total of Rs. 8.38 crores in the economy of Rajasthan. The yields of CFLDs surpassed the results of Rajasthan by 7.02q/ha and national yield by 7.53q/ha. The identified technology gaps attributed to the dissimilarity in soil fertility status and weather conditions. Hence, the adoption of the integrated crop management practices could fulfill these extension gaps. Scientists of KVKs should adopt location-specific extension methodologies for approaching partner farmers. Moreover, a lower value of the technology index indicates greater technology feasibility in the particular district.

***Key words*:** Chickpea, Cluster front line demonstrations, Gap, Income, Yield.

INTRODUCTION

In India, food security is one of the prominent issues. However, scientists are thinking beyond food security viz. nutritional security wherein pulses play a vital role. They have high protein content and therefore play a crucial role in diet of the vast majority of India's low-income and vegetarian population (Kumar *et al.* 2019). Pulse crops were reported to grow on more than 29 million hectares of land and yield just 25.23 MillionTonnes (MT) with average productivity of 841kg/ha during 2017-18. Under pulses, Chickpea contributes maximum with the production of 11.23 MT at a record productivity level of 1063 kg/ha in an area of 10.56 million hectares. More than 90% of Chickpea production contributed by Madhya Pradesh (4.60 MT), Maharashtra (1.78 MT), Rajasthan (1.67 MT), Karnataka (0.72 MT), Andhra Pradesh (0.59 MT), Uttar Pradesh (0.58 MT), and Gujarat (0.37 MT). Among pulses, the Chickpea plays a vital role in improving soil fertility since it can survive in drought conditions. Rajasthan is among the significant contributors to Chickpea production. Despite the large area, the full potentials of improved varieties and technologies are yet to be harnessed. Farmers' methods are no longer viable because they demonstrate a considerable yield difference instead of scientific production technologies. Hence, there is a need to increase the adoption of scientific production technology. A constant effortis needed to bridge this gap by showing improved production technology (Sumathi, 2012). The CFLDs area modern approach to having a direct interface between the researcher and the farmer to transmit their existing technology to gain immediate feedback from the farming community. The study shows that in 2015-16, the average results of CFLDs in Rajasthan were 14.87q/ha, which was 2.91q/ha higher than farmers' practices. The yield of Chickpea was enhanced by 3.76q/hawas observed in 2016-17, where CFLDs yield was 16.30q/ha (Meena *et al.*, 2017). However, in 2017-18, the CFLDs yield was 19.91q/ha, which shows a higher yield of 4.59q/ha. However, during 2018-19, the average result of CFLDs was 18.45 q/ha,which offers an increaseof 4.04q/ha yields(Annual Report, ATARI, Jodhpur, 2017-18 and 2018-19). Therefore, a study was undertaken to delineate the technological intervention gap, economics, and income augmentation from 2015 to 2020.

MATERIALS AND METHODS

The KVKs of Rajasthan laid out the CFLDs on Chickpea from 2015 to 2020. The CFLDs were

conducted in cluster mode by following a participatory approach. Critical inputs, i.e., the seed of improved varieties, bio-fertilizers, soil ameliorates, herbicide, micro-nutrients, integrated pest & disease management,etc., of maximum Rs.9000/ha provided by KVKs. Many extension activities, i.e., organization of field days, monitoring for crops, etc.,for creating the informal contact so that learnings can take place for acceptance and broader adoption among other farmers. Partner farmers themselves applied fertilizers to attain the potential yields. The scientists from KVKs have frequently visited the fields to resolve the problems in the fields. An area of 0.40 ha for each CFLD was allocated for partner farmers.Hence, a complete package of the practicesof Chickpea was demonstrated in the farmers' fields. The data were collected from 13134 partner farmers. The yield data collected from established technology and farmers' practices by random crop cutting method. The technology gap, extension gap, and technology index of Chickpea were computed. Data were analyzed using simple statistical tools.The gross return, the net return, cost of cultivation, and benefit-cost ratio were also calculated. The methodology used by Yadav et al. 2004, was adopted for computing extension gaps, technology gaps, and technological index.

Extension gap = Demonstration yield-Farmer yield

Technology gap = Potential yield-Demonstration yield

$$\text{Technology index} = \frac{\text{Potential yield} - \text{Demonstration yield}}{\text{Potential yield}} \times 100$$

RESULTS AND DISCUSSION

Chickpea yields and gap optimization

Study shows (Table 1) an average yields of Chickpea under CFLDs, which was observed in the range from 13.46 q/ha (Barmer I) to 23.41q/ha (Baran) in Rajasthan state. However, the range of yields under farmers' practices was from 9.51 q/ha to 18.95 q/ha. In a nutshell, the average grain yield of Chickpea under CFLDs was 17.94 q/ha in the demonstrated fields compared to farmers' practice (13.71 q/ha). This yield was 36.81 % higher than the farmers' yields. Figure-1 clearly shows that percent yield under CFLD was higher during 2015-16. Since the CFLDs were conducted by KVKs during 2015-16 under NFSM. Farmer were growing old varieties hence a significant enhancement in yield was depicted (52.60 %) by introducing new varieties in the area. In addition to CFLDs, KVK also organized field days and extension activities in the cluster of villages to make farmers aware to grow improved chickpea varieties or to purchase from reliable sources. Some farmers kept the seed for the next year hence the yield of even under control was higher than 2015-16 in the forthcoming years. KVKs were classified based on the results observed in the fields in five years (Table 2). Out of 43 KVKs in Rajasthan state, 6 KVKs, namely Sirohi, followed by Banswara, Baran, Ajmer, Bhilwara, and Jodhpur-II, had increased yield more than 5.42 q/ha. However, 7 KVKs, namely Hanumangarh II, followed by Bikaner II, Pali,Sriganganagar, Bharatpur, Hanumangarh I and Udaipur II, were identified where yields were increased, but below 3.04 q/ha. The remaining 30 KVKs were in the range of 3.04 to 5.42q/ha compared to the local (Tables 1 and 2).

Extension gap, technology gap, and technology index in chickpea

The extension gap is defined as the difference between demonstration yield and the yield of farmers' practice. The extension gap was found as 4.23 q/ha.The highest extension gap was reported in the Sirohi district (8.09 q/ha) of Rajasthan, followed by Banswara (6.73 q/ha) and Baran (6.44 q/ha) district. Relatively, a high extension gap was showed in Ajmer (6.24 q/ha), Bhilwara (5.74 q/ha), Jodhpur-II (5.6 q/ha), and Jhalawar (5.21 q /ha) district. Similar findings are also reported by Kumar (2021). To reduce the extension gap, we need to educate and motivate partner farmers to implement the improved production technologies of Chickpea. Much effort needs to be made by ICAR, Government institutes, SAUs, and KVKs through various extension programs to disseminate the improved practices. More extension gaps indicate the high acceptance of advanced technologies. The technology gap is the output of differences between potential yield and demonstration yield. From 2015 to 2000, the technological gap was observed as 5.75 q/ha. The technology gap may be attributed to the dissimilarity in the soil fertility status and weather conditions, and similar findings were found by Kumar (2021) and Mitra &Samajdar (2010). Less technology gap revealed better adaptability of crop variety in a particular area; among all CFLDs in Pali (- 0.39) and Tonk (- 0.52), a negative gap was observed. These KVKs demonstrated the varieties like RSG-895, RSG-974, and GNG-1581, GNG-1958 performed well and showed lesser technological gaps. The primary reason behind relatively high yield performance was good rainfall in the last week of September or 1st week of October. It means in that area, demonstration yield was more than potential yield and variety shows better adaptability.InSirohi, a significantly less technology gap (0.13 q/ha) was observed, followed by Nagore-II

Table 1. Chickpea yield and gaps minimized during 2015-2020 in Rajasthan, India (n=13134).

Sl. No.	KVKs	Number of CFLDs	Yield gap minimized (q/ha)						
			Farmers practices (q/ha)	CFLDs (q/ha)	Yield increase (q/ha)	Yield increase (%)	Extension gap (q/ha)	Technology gap (q/ha)	Technology index (%)
1.	Jodhpur-I	199.00	13.80	18.39	4.59	32.71	4.59	8.41	31.38
2.	Jodhpur-II	74.00	15.60	21.20	5.60	35.94	5.60	2.80	11.66
3.	Barmer-I	177.00	9.51	13.46	3.95	46.16	3.95	8.87	39.72
4.	Barmer-II	92.00	10.95	14.50	3.55	32.53	3.55	12.30	45.89
5.	Hanumangarh-I	400.00	16.40	18.85	2.45	14.99	2.45	5.22	21.68
6.	Hanumangarh-II	150.00	15.17	18.20	3.03	20.01	3.03	8.60	32.08
7.	Sriganganagar	414.00	14.23	17.01	2.78	20.76	2.78	8.67	33.76
8.	Churu-I	425.00	9.93	14.27	4.34	50.59	4.34	10.29	41.89
9.	Churu-1I	125.00	11.38	14.54	3.16	28.77	3.16	10.86	42.75
10.	Bikaner-I	350.00	13.94	17.99	4.05	29.46	4.05	6.21	25.66
11.	Bikaner-II	125.00	13.25	16.14	2.89	21.81	2.89	7.86	32.75
12.	Jaisalmer-I	135.00	10.13	14.06	3.93	41.45	3.93	9.54	40.42
13.	Jaisalmer-II	50.00	12.52	16.86	4.34	34.66	4.34	5.94	26.05
14.	Sikar	415.00	14.50	17.80	3.30	24.99	3.3	5.96	25.08
15.	Nagaur-I	332.00	12.97	16.27	3.30	25.89	3.3	6.49	28.51
16.	Nagaur-II	125.00	14.32	18.92	4.60	26.32	4.6	0.58	2.97
17.	Jhunjhunu	425.00	11.15	14.29	3.14	31.62	3.14	9.47	39.85
18.	Jalore	150.00	11.73	16.13	4.40	37.89	4.4	4.87	23.19
19.	Pali	252.00	12.00	14.89	2.89	23.81	2.89	-0.39	-2.68
20.	Sirohi	359.00	10.38	18.47	8.09	96.57	8.09	0.13	0.69
21.	Tonk	325.00	15.99	20.92	4.93	31.72	4.93	-0.52	-2.54
22.	Jaipur-I	388.00	14.65	19.11	4.46	30.76	4.46	3.09	13.91
23.	Jaipur-II	200.00	15.33	19.41	4.08	26.62	4.08	4.59	19.12
24.	Ajmer	305.00	11.62	17.86	6.24	67.51	6.24	6.14	25.58
25.	Dausa	590.00	15.06	19.07	4.01	39.12	4.01	5.25	21.58
26.	Alwar-I	239.00	14.77	18.77	4.00	29.56	4	5.23	21.79
27.	Alwar-II	102.00	18.95	22.88	3.93	20.80	3.93	2.52	9.92
28.	Dholpur	454.00	16.32	20.43	4.11	28.17	4.11	5.81	22.14
29.	Karauli	415.00	14.27	18.90	4.63	36.70	4.63	6.22	24.76
30.	Bharatpur	350.00	12.32	15.08	2.76	22.62	2.76	4.17	21.66
31.	Bhilwara	400.00	14.04	19.78	5.74	46.50	5.74	4.78	19.46
32.	Chittorgarh	481.00	16.42	20.95	4.53	27.86	4.53	0.81	3.72
33.	Rajsamand	363.00	14.47	19.32	4.85	35.20	4.85	5.80	23.08
34.	Pratapgarh	350.00	13.84	18.02	4.18	30.76	4.18	6.68	27.04
35.	Udaipur-I	389.00	11.84	16.21	4.37	37.07	4.37	6.35	28.14
36.	Udaipur-II	50.00	14.80	16.90	2.10	14.19	2.1	9.90	36.94
37.	Banswara	325.00	11.04	17.77	6.73	62.95	6.73	5.79	24.57
38.	Dungarpur	669.00	9.92	14.24	4.32	46.38	4.32	10.32	42.01
39.	Kota	455.00	16.90	20.51	3.61	21.83	3.61	5.81	22.07
40.	Bundi	345.00	16.60	20.25	3.65	25.24	3.65	5.39	21.02
41.	Jhalawar	475.00	14.05	19.26	5.21	39.01	5.21	3.14	14.01
42.	Sawai Madhopur	390.00	15.43	20.15	4.72	31.86	4.72	5.53	21.53
43.	Baran	300.00	16.97	23.41	6.44	37.71	6.44	1.79	7.10
	Total	**13134**	-	-	-	-	-	-	-
	Average	-	**13.71**	**17.94**	**4.23**	**34.12**	**4.23**	**5.75**	**23.53**

Source: Primary data collected from 2015 to 2020.

(0.58 q/ha) and Chittorgarh (0.81 q/ha). The technology index indicates the level of feasibility of demonstrated technology in farmers' fields. The lowest value (-2.68 %) of the technology index was observed in the Pali district, followed by Tonk (-2.54 %). In Sirohi, Nagur-II, and Chittorgarh district, low indexes were observed and reflect high feasibility on farmers' fields. These KVKs obtained the yield more than the potential yields. The highest value of the technology index was

Table 2. Ranking of KVKs based on the yield enhancement through CFLDs on Chickpea (in q/ha).

Sl.No.	Yield (q/ha)	*f*	%
1.	>5.42	6	13.95
2.	3.04 to 5.42	30	69.77
3.	<3.04	7	16.28

Table 3. Economics of Chickpea production in Rajasthan, India (n=13134).

Sl. No.	KVKs	Area (ha)	CFLDs	Economics of FP (Rs/ha)				Economics of CFLDs (Rs/ha)				Total income enhancement
				Gross cost	Gross return	Net return	B.C. ratio	Gross cost	Gross return	Net return	B.C. ratio	
1.	Jodhpur-I	80.00	199.00	23,741.67	60,431.25	36,689.58	2.53	26,450.00	84,674.04	58,224.04	3.20	16,64,192.50
2.	Jodhpur-II	40.00	74.00	24,222.50	74,035.50	49,813.00	3.06	26,355.00	99,929.06	73,574.06	3.79	9,50,442.50
3.	Barmer-I	127.40	177.00	24,800.50	44,907.50	20,107.00	1.82	27,566.50	57,817.63	30,251.13	2.12	13,27,617.90
4.	Barmer-II	60.00	92.00	27,897.33	50,758.79	22,861.46	1.80	30,635.00	66,799.75	36,165.25	2.17	7,98,227.50
5.	Hanumangarh-I	160.00	400.00	25,347.80	72,680.20	47,332.40	2.87	27,135.20	83,218.70	55,883.50	3.07	14,41,000.00
6.	Hanumangarh-II	60.00	150.00	17,708.67	63,700.00	31,414.33	3.60	20,551.00	76,447.00	38,026.00	3.72	3,96,700.00
7.	Sriganganagar	178.00	414.00	24,134.70	57,216.10	33,081.40	2.42	26,592.17	69,331.10	42,738.93	2.69	15,72,449.60
8.	Churu-I	170.00	425.00	20,565.00	45,316.00	27,050.20	2.19	24,350.40	71,405.40	47,254.80	2.91	31,83,810.00
9.	Churu-1I	50.00	125.00	22,060.00	55,948.56	33,888.56	2.51	23,960.00	71,497.25	47,537.25	2.95	6,53,427.50
10.	Bikaner-I	140.00	350.00	26,460.00	56,326.58	29,866.58	2.21	28,200.00	73,435.42	45,235.42	2.69	21,07,400.00
11.	Bikaner-II	50.00	125.00	28,430.00	62,936.25	34,506.25	2.21	30,492.50	76,668.00	46,175.50	2.51	5,81,380.00
12.	Jaisalmer-I	58.00	135.00	21,296.67	58,450.00	36,630.42	2.84	21,966.67	70,732.50	48,765.83	3.28	7,81,157.50
13.	Jaisalmer-II	20.00	50.00	24,060.00	61,010.63	36,950.63	2.54	28,228.00	82,192.50	53,964.50	2.91	3,40,277.50
14.	Sikar	166.00	415.00	28,200.00	76,123.87	47,923.87	2.68	30,830.00	87,870.47	57,053.80	2.84	13,73,035.33
15.	Nagaur-I	148.80	332.00	23,139.00	64,998.75	41,859.75	2.82	23,959.60	82,131.57	58,171.97	3.43	21,79,817.60
16.	Nagaur-II	50.00	125.00	27,991.67	77,866.75	49,875.08	2.78	30,448.60	1,00,953.03	70,504.43	3.31	10,32,760.00
17.	Jhunjhunu	170.00	425.00	28,725.00	52,089.20	23,364.20	1.82	30,454.00	59,441.60	28,987.60	1.95	10,00,640.00
18.	Jalore	60.00	150.00	22,500.00	52,560.25	29,766.50	2.34	24,283.33	73,707.38	49,424.05	3.03	11,39,384.00
19.	Pali	109.00	252.00	19,860.50	44,246.25	24,385.75	2.23	19,980.06	56,196.43	38,716.37	3.06	15,74,124.10
20.	Sirohi	138.00	359.00	23,165.90	64,528.50	32,882.60	2.81	25,414.00	85,488.30	60,074.30	3.37	35,06,570.00
21.	Tonk	140.00	325.00	21,049.60	81,418.50	60,368.90	3.64	23,920.00	1,05,695.90	81,775.90	4.18	29,92,600.00
22.	Jaipur-I	160.00	388.00	22,501.07	68,659.90	46,158.83	3.05	24,486.67	89,528.37	65,175.03	3.65	30,74,843.33
23.	Jaipur-II	80.00	200.00	23,020.00	66,599.17	43,579.17	2.90	26,146.00	83,950.08	57,804.08	3.22	11,27,325.00
24.	Ajmer	150.00	305.00	23,277.73	60,951.47	37,670.13	2.66	26,054.20	84,294.90	58,240.70	3.25	25,32,996.67
25.	Dausa	260.00	590.00	33,382.00	72,122.87	38,740.87	2.20	33,039.20	83,745.15	50,705.95	2.54	35,41,176.67
26.	Alwar-I	146.25	239.00	19,864.90	69,244.40	51,887.40	2.97	21,771.00	88,023.90	66,252.90	3.43	17,86,433.75
27.	Alwar-II	50.00	102.00	22,470.17	89,539.71	67,069.54	3.99	24,702.82	1,08,539.10	83,836.29	4.39	8,29,803.46
28.	Dholpur	176.00	454.00	25,403.00	78,925.05	53,522.05	3.11	28,062.40	93,511.00	65,448.60	3.33	22,16,695.00
29.	Karauli	166.00	415.00	25,992.20	70,657.25	44,665.05	2.77	29,250.60	89,674.38	60,423.78	3.14	24,47,718.75
30.	Bharatpur	140.00	350.00	20,143.75	55,667.75	35,524.00	2.78	22,218.75	67,219.35	45,005.30	3.06	12,65,152.00
31.	Bhilwara	160.00	400.00	22,060.00	69,530.60	47,470.80	3.21	25,180.00	93,342.20	68,262.20	3.77	32,92,710.00
32.	Chittorgarh	190.00	481.00	24,155.72	76,625.20	52,469.28	2.98	28,006.67	97,750.70	69,641.33	3.28	32,90,520.00
33.	Rajsamand	150.00	363.00	19,400.00	71,223.00	51,823.00	3.64	21,657.00	82,649.97	66,992.97	3.72	22,49,843.33
34.	Pratapgarh	140.00	350.00	20,959.00	56,479.50	35,520.50	2.71	23,609.50	73,468.00	49,858.50	3.11	21,75,490.00
35.	Udaipur-I	170.00	389.00	22,755.00	51,484.40	28,729.40	1.77	26,341.80	70,447.00	44,105.20	2.11	26,00,280.00
36.	Udaipur-II	20.00	50.00	31,000.00	66,600.00	35,600.00	2.15	32,500.00	87,880.00	55,380.00	2.70	3,95,600.00
37.	Banswara	130.00	325.00	28,320.00	46,498.55	18,178.55	1.63	31,560.00	74,341.80	42,781.80	2.35	31,66,525.00
38.	Dungarpur	140.00	669.00	23,616.00	43,300.00	19,684.00	1.89	26,867.00	69,191.00	42,324.00	2.71	29,80,450.00
39.	Kota	188.00	455.00	26,207.60	81,613.60	55,406.00	3.14	28,164.80	98,720.70	70,555.90	3.51	24,75,450.00
40.	Bundi	160.00	345.00	27,281.20	80,269.20	52,988.00	2.92	29,608.80	97,367.20	67,758.40	3.27	23,16,290.00
41.	Jhalawar	190.00	475.00	24,165.40	61,573.48	37,025.28	2.58	25,746.90	83,619.75	57,472.76	3.26	39,48,510.60
42.	Sawai Madhopur	156.00	390.00	23,949.84	76,503.94	52,553.90	3.19	26,451.00	96,621.64	70,170.64	3.66	28,16,527.05
43.	Baran	120.00	300.00	24,129.50	87,229.56	63,099.94	3.45	25,020.04	1,14,697.90	89,677.86	4.37	27,51,316.40
	Total	**5417.45**	**13134**	-	-	-	-	-	-	-	-	**83878670.54**
	Average	-	-	**24,172.34**	**64,624.37**	**39,999.63**	**2.68**	**26,470.17**	**73,586.54**	**56,194.86**	**3.14**	-

Source: Primary data collected from 2015 to 2020.

reported in CFLDs under Barmer-II (45.89 %). CFLDs under Barmer-II, irrigation was not available hence showed lesser yields. The observed technology difference can be attributed to dissimilarity in soil fertility status, rainfall distribution, disease, insect, pest infestations, and weed intensity and the change in cluster front line demonstration sites' locations. The technology index demonstrates the viability of the variety at the farmer's field; lowering the technology index's value indicated greater technology feasibility. It shows the efficacy of good performance of relevant interventions or technologies demonstrated in farmer's field. As a result, this could increase in yield of Chickpea under the different agro-ecological situation of Rajasthan. These findings corroborate the findings reported by Meena (2017) and Lakshmi *et al.* (2017).

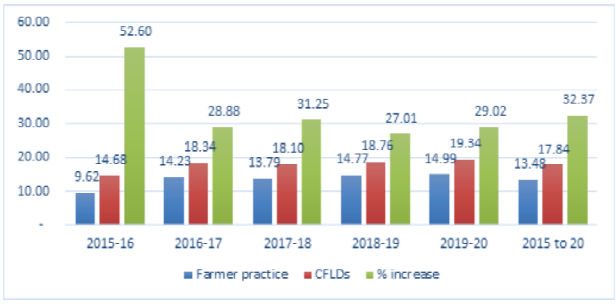

Fig. 1. Yields of Chickpea (q/ha) during 2015-2020 in Rajasthan, India (n=13134).
Source: Primary data collected from 2015 to 2020.

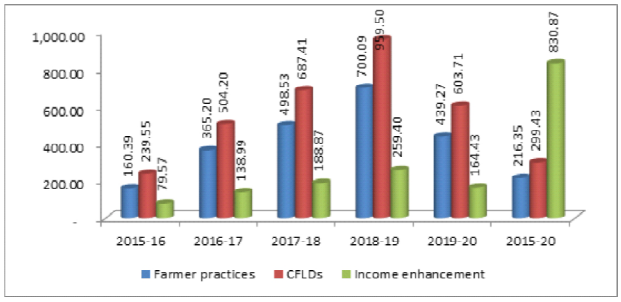

Fig. 2. Income enrichment by conductance of CFLDs on Chickpea in Rajasthan, (in lakh).
Source: Primary data collected from 2015 to 2020.

Economics of chickpea production

The economic performance of Chickpea under CFLDs is depicted in table-3. During the five years, itrevealed that the Chickpea recorded a higher net return from recommended practices. Under CFLDs, net return was Rs 56,194.86 /ha than farmers' practices (Rs. 39,999.63 /ha).Hence, a total of Rs.8.38 crores have been added through these CFLDs in the states' economy in the last five years (2015-2020). Nevertheless, average of the previous five years under all KVKs, the benefit-cost ratio of CFLDs was 1:3.14 while 1:2.68 in farmers' practices was. The higher net returns and B:C ratio in chickpea demonstration might be due to the higher grain yield and better market pricing.

CONCLUSION

The cluster front line demonstrations on Chickpea showed asignificant and positive result, which provided opportunities to the KVKs for demonstrating the latest production technologies. The productivity gained under CFLDs over existing Chickpea cultivation practices has created greater awareness and motivation amongst other fellow farmers to adopt suitable production technology of chickpea. There exists a wide gap in the potential yields, demonstration yields & farmers' plot yields due to technological (5.75 q/ha) and extension gaps (4.23 q/ha). The study emphasizes the dissemination of location-specific crop management, improved technologies embedded with high-yielding varieties to minimize these gaps and improve pulse productivity & profitability in Rajasthan. Moreover, the state's extension functionaries strictly focus on disseminating the proven pulse production technologies in chickpea production systems.

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Antioxidant activity of different mungbean genotypes in relation to phenolic acids, flavonoids and seed coat colour

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ABSTRACT

The diversity of mungbean in terms of phenolic acid, flavonoids and antioxidants was studied using thirty mungbean genotypes. Significant (P<0.05) differences were found in the contents of both bound and free phenolic acids e.g. syringic acid, p-coumaric acid, caffeic acid and ferrulic acids, comprising about 90% of total phenolic acids. The wild accession *V. sylvestris* recorded maximum free phenolic caffeic acid in seeds. Antioxidant activity in seed determined based on radical scavenging capacity revealed. a range of antioxidant activity with maximum >30 µmol g^{-1} recorded in three wild accessions *V. sylvestris*, *V. umbellata* and *V.trilobata* and lowest in ML 818 (28.13 µmol g^{-1}) using DPPH method. However, with ABTS method, minimum activity was observed in DGGV 2 . Flavonoids and phenolic acids both act as antioxidant in mungbean and thus higher amount enhances the nutrive value of mungbean. The results showed that total flavonoids content (TFC) and and total phenolic acid (TPC) were significantly (P<0.001) correlated with antioxidant activities with correlation coefficients of 0.875 and 0.708. suggesting these mungbean cultivars are rich in balanced nutrients and should be considered as potential sources of natural antioxidants. It was observed that dark coloured seed coat genotype or yellow seed contained higher antioxidant activity than normal green seeds of mungbean . The dull green or pale brownish mung wild accession *Vigna umbellata* had the highest value of total flavonoid (TFC) and total phenolic acid (TPC) and exhibited highest antioxidant activity followed by yellow sona mung. The results indicated that seeds of some of the wild accessions and landraces are very nutrient dense with high antioxidant activities which could be explored for developing superior mungbean varieties.

Key words: Antioxidants, Flavonoids, Mungbean nutritive value, Phenolic acid

Mungbean (*Vigna radiata* L. Wilczek), is one of the important pulse crops, which belongs to genus *Vigna*, species *radiata* and family Leguminoseae (Shil and Bandopadhyay, 2007). The crop is predominantly grown for edible part that is its dried seeds and is well adapted to tropics as a summer and rainfed ecosystem. This crop fixes atmospheric nitrogen through symbiotic association with *Rhizobium* and as a result the crop derives benefits in terms of naturally fixed nitrogen and also increases fertility of soil for succeeding crops. Mungbean seeds are rich in protein, carbohydrates, folate, minerals such as iron, zinc, and magnesium., amino acids, and wide range of biologically active compounds (Anwar *et al*., 2007; Tang *et al*., 2014; Dahiya *et al*, 2015). In addition, the phytochemicals, saponins, and tannins found in mungbean possess antioxidant and anti-carcinogenic effects.

Importance of this crop is realized due to its unique biochemical properties; nutritive values of mungbean are superior as compared to other pulses. In spite of its importance as food and feed, very little attention has been paid to its qualitative improvement. For efficient utilization of germplasm, it is important to investigate the extent of variability in its biochemical composition and its magnitude for the determination of success of a breeding program (Smith et al., 1991). An initial step in a breeding program is the assembly of germplasm with a wide range of genetic variability in terms its chemical composition such as protein, dietary fibres, amino acids, phenolic acids, resistant starch etc. however the chemical properties of mungbean genotypes and their diversity is little explored. The biochemical composition of mungbean is considered to be important as it fulfils all the nutrient requirement of human being except sulphur

containing amino acids. The seeds of mungbean provide excellent source of protein, dietary fiber, minerals, vitamins, and significant amounts of bioactive compounds, including polyphenols, polysaccharides, and peptides. Therefore, mungbean is becoming a popular functional food in promoting good health. In mungbean, vitexin and isovitexin are the major polyphenols, However, there are still important knowledge gaps with regards to its bioactive compounds and biological activities of the mungbean. The polyphenols vitexin and isovitexin are the main functional components which have drawn interest in terms of their health benefits however, further research is also needed to unravel other main functional components relevant to the nutritional benefits and highlight the synergistic multi-component effects of mung bean on biological functions.

Some bioactive compounds of mungbean are considered anti-nutritional factors (ANF) such as phytic acids, phenolic acids, raffinose group of oligosaccharides and flavnols. They are called anti-nutritional components as they cause flatulence, act as trypsin inhibitors and chelating agents that bind with essential cations such as zinc, iron magnesium and thus lower the bioavailability of minerals in human being. However, at the same time these ANFs have certain beneficial effects or therapeutic uses also. One of the strategies to lower ANFs in seeds is proper processing such as sprouting, soaking, dehulling, autoclaving and milling etc (Mubarak., 2005).

There is great diversity in mungbean seed coat colour and also significant differences in phenolic acid and flavonoids in seeds. Some genotypes bear dark brown pod colour while some bear blackish brown pod colour and produce brown coloured pods. Seed coat lustre (shining vs medium shining), colour and seed size (medium vs. bold vs. medium bold) also vary among genotypes at mature seed stage.

Comparative biochemical studies on health promoting phytochemicals at population level are important from the point of view of developing breeding strategies. There is an urgent need to develop a compositional and functional database on mungbean quality. Therefore, the investigation was undertaken with an objective to establish existing biochemical variation in terms of phenolic acid and flavonoids content in relation to seed coat colour and also to correlate their chemical properties as antioxidants.

MATERIALS AND METHODS

Pure seeds of thirty mungbean germplasm including wild accessions, landraces and released varieties was procured from ICAR-Indian Institute of Pulses Research, Kanpur. All these were assessed for phenolic acid and flavonoids profiles, however, limited genotypes having different seed coat colour were evaluated for antioxidant activity to understand any association of biochemical changes with seed coat colour. Since green seeded mungbean is abundantly available, four genotypes (ML 818, SML 668, IPM 205-7 and IPM 02-3) with green seed colour were chosen and one dark-green coloured seed coat genotype EC 398889 , one yellow seed Sona mung and pale brownish *V.umbellata* were also evaluated for comparison of antioxidant activity.

Extraction and estimation of total phenolic content

Total phenols were extracted and estimated as described by Swain and Hills (1959). Mungbean flour (10 g) and 100 mL of 70% ethanol were mixed and extracted twice for 2 h at room temperature. After vacuum filtration, the supernatants were combined and concentrated under reduced pressure in a rotary evaporator at 50 °C. After freeze-drying, sample powder was stored at "20 °C until analysis. The previously reported (Yen and Chen, 1995) Folin–Ciocalteu method was used to evaluate TPC. Briefly, 50 ìl of the extract and 5 ml of distilled water were mixed in a test tube, and 500 ìl of 1 mol l^{-1} Folin–Ciocalteu reagent and 500 ìl of a 20% (w/v) Na_2CO_3 solution were injected into the tube. After thorough mixing, the tube was allowed to stand for 60 min at room temperature. Finally, the absorbance was measured at 765 nm (Beckman, UK). Quantification was performed with respect to a standard curve of gallic acid. Contents were reported in mg gallic acid equivalent (GAE) per gram.

Extraction of free phenolic acid

Free phenolic acids were extracted following López et al. (Lopez et al., 2013) with modification. One gram of mungbean flour and 20 ml of 70% chilled ethanol were mixed in a tube. Tubes containing samples were shaken on a shaker for 10 min at room temperature. After centrifugation at 2500 rpm for 10 min, the supernatant was transferred to a new tube and the residue was extracted once more. Supernatants were combined, evaporated at 45 °C to less than 5 ml, and diluted with distilled water to 10 ml. Extracts were stored at -20 °C.

Extraction of bound phenolic acid

Bound phenolic acids were extracted following a previously reported method (Zhang *et al.,* 2012).

Fifteen milliliters of distilled water, 5 ml of NaOH (6 mol l-1) and the residue after the extraction of free phenolic compounds were mixed in a test tube and stirred for approximately 16 h at room temperature. The solution was then adjusted to pH 2.0 and the liberated phenolic acids were extracted three times with 15 mL of a mixture of cold diethyl ether (DE) and ethyl acetate (EA,1:1 v/v). The DE/EA layers were combined and evaporated to dryness and the residue was dissolved in 1.5 mL of methanol. Acid hydrolysis was then performed by addition of 2.5 mL of concentrated 12 mol l^{-1} HCl to the test tube and incubation in a water bath at 85 °C for 30 min after completion of the alkaline hydrolysis. The sample was cooled and adjusted to pH 2.0, with DE/EA extraction performed in the same manner as for alkaline hydrolysis.

Determination of total flavonoid content (TFC)

Mungbean flour (0.5 g) and 20 ml of 70% methanol were mixed and shaken in a water bath at 70°C for 2 h. The solution was centrifuged at 1500 rpm for 10 min. One milliliter of supernatant was dried in a freeze drier. Before tests were performed, methanol was used to dissolve the dried sample. An 0.5 ml appropriate dilution of extract, 1.5 mL of 95% ethanol, 0.1 ml of 10% aluminum chloride ($AlCl_3$) hexahydrate, 0.1 ml of 1 mol l^{-1} potassium acetate (CH_3COOK), and 2.8 mL of deionized water were mixed. Before the absorbance of the reaction mixture was measured at 415 nm against a deionized water blank on a Beckman Spectrophotometer, the mixture was incubated at room temperature for 40 min. Total flavonoid content was determined on the basis of a calibration curve of authentic rutin (Qin et al 2010). Alternatively, determination of total flavonoid content (TFC) in seed samples was also done using the procedure as developed by Lee *et al*. (2011) with a minor modification. The sample extracts and distilled water at ratio of 1:5 v/v were thoroughly mixed in a tube. Sodium nitrite solution (1:20 v/v) was added to the sample and mixed. After incubation for 6 min, 50 µl aluminum chloride solution was added and incubated for 5 min. Prior to addition of distilled water, sodium hydroxide (1000 µl) was added to the mixed solution. After being thoroughly mixed, absorbance values were measured at 510 nm using a spectrophotometer. Total flavonoid contents in the sample were expressed as catechin equivalents per gram of the sample (mg CE/ g).

Determination of individual phenolic acid

An Agilent-1100 UV detector and an Agilent TC-C18 (250.0 mm × 4.6 mm, 5 ìm) were used to analyze individual phenolic acids. The wavelengths of the detector were set at 280 and 320 nm. The ratio of the mobile phase was as follows: solvent A (HPLC water containing 0.05% TFA) and solvent B (acetonitrile: MeOH:TFA = 30:10:0.05). The gradient elution was programmed as follows: from 10% to 12% B over 16 min, from 12% to 38% B over 9 min, from 38% to 70% B over 7 min,from 70% to 85% B over 8 min, and from 85% to 100% B over 10 min. The flow rate was fixed at 1.0 mL min-1 and the injection volume was 20 ìL. Each phenolic acid was quantified according to its calibration curve.

Seed analysis for antioxidant composition

Extraction and estimation of DPPH radical scavenging activity:

Scavenging activity on DPPH free radicals was assessed according to the method of Gyamf et al. (1999). Mungbean seed powder (100 mg) was extracted with 2 ml methanol. For estimation , 1 ml of supernatant was added to 3 ml of 0.1 mM DPPH and kept in dark for 30 min. Absorbance was read at 518 nm. DPPH radical-scavenging activity was calculated using the relation;

DPPH% inhibition = (A blank- A sample)/A blank) × 100

A=absorbance at 518 nm.

An alternate reported method was used to quantify DPPH radical scavenging activity (Yen and Chen, 1995). DPPH (100 ìmol L^{-1}) was dissolved in 96% ethanol. The DPPH solution (1 ml) and 1 ml of the extract solution were mixed. After being shaken, the mixture was let stand at room temperature in the dark for 10 min. Finally, the decrease in absorbance of the resulting solution was measured at 517 nm after 10 min. The results are reported in ìmol of Trolox equivalents (TE) per gram

ABTS+ assay

A reported method was used to identify the ABTS+ radical scavenging activity (Yao et al., 2010). Briefly, redistilled water was used to dissolve ABTS+ to a concentration of 7 ìmol L-1. An ABTS+ radical cation was produced by reacting ABTS+ stock solution with 2.45 mmol L-1 potassium persulfate and storage at room temperature for 16 h in the dark. The resulting solution containing the ABTS + solution was diluted with redistilled water to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C. A reagent blank reading was then taken. Before the absorbance was measured exactly 6 min after initial mixing, 3.0 mL of diluted ABTS+ solution (A 734 nm = 0.70 ± 0.02) was

added to 30 ìL of the extracts or Trolox (prepared in DMSO for use as standard). The results are expressed as ìmol of Trolox equivalents per gram. All determinations were performed in triplicate.

Statistical analysis

To determine significant differences among all genotypes the one way analysis of variance was done. Data were subjected to analysis of variance for each year and combined over both years and tested for significance using SAS (version 9.1.3) and correlations between TPC, TFC, and ABTS+ were identified using Spearman's correlation (SPSS 17.0). Correlations were considered highly significant at $P < 0.01$. Pearson's correlation coefficients were estimated with SAS 9.3.1. The mixed model was used to identify significant differences among different seed coat colour. All values were expressed as mean ± SEm. One-way analysis of variance (ANOVA) at $p<0.05$ was used for the analytical variation. Least significant difference (LSD) test as multiple comparison methods was used to determine differences between means of the sample with a level of significance of 0.05. Means and standard deviations were calculated. Analysis of variance was used to determine the variation between the samples. The critical difference (CD) at 5% where the F-ratio was significant was calculated by the CPCS computer program I .Data were expressed as mean ± standard deviation (SD) for triplicates. Biochemical analysis between control and test was subjected to statistical analysis by t-test at 5 % level of significance

RESULTS AND DISCUSSION

Mungbean contains many secondary metabolites such as phenolic acids and flavonoid. Phenolic acid represents the most common form of phenolic componds and constitutes one of the major and most complex groups of phytochemicals in grain. Both phenolic acids and flavonoides contribute to the antioxidant activity of mungbean. By using HPLC, four bound phenolic acids, p-coumaric acid,ferulic acid, syringic acid and caffeic acid were found in mungbean seeds (Table 1) and two free phenolic acids (caffeic acid and ferulic acid) detected in mungbeans under test (Table 1).

The contents of individual phenolic acids in different mungbean genotypes are shown in Table 1. The average total content of free phenolic acids (the sum of the two individual i.e caffeic acid and ferrulic acids) in the mean of 30 mungbean genotypes was 291.67 µg g^{-1}, comprising about 14.5% of total phenolic acids (TPC) as determined in mungbean genotypes. There were wide variation in free phenolic acids both caffeic and ferrulic acids ranging from (187.5 to 258.63 µg g-1) and (6.87-9.21 µg g-1) respectively. The wild accession *V. sylvestris* recorded maximum free phenolic caffeic acid in seeds .Significant ($P<0.05$) differences were found among all mungbean genotypes in the contents of both bound and free phenolic acids. The total and bound phenolic acids (the sum of the four individual phenolic acid e,g. syringic acid, p-coumaric acid, caffeic acid and ferrulic acids) was 1796.3 µg g-1), comprising about 90% of total phenolic acids (TPC). Caffeic acid was the dominant phenolic acid in both free and bound forms in mungbean tested from diverse sources.

Phenolic acid in mungbean seeds act as antioxidant properties. These are the substances which neutralize the free radicals causing oxidative stress are known as antioxidants. Reactive oxygen species such as hydroxyl radicle (*OH), hydrogen peroxide (H_2O_2), superoxide (O_2-) are major sources of oxidative stress in cells, damaging proteins, lipids and DNA (Sharma *et al*., 2012)). This oxidative stress causes induced ageing and several degenerative diseases such as heart disease, cataracts, cognitive dysfunction and cancer (Tan *et al* . 2018)). Natural compounds such as mungbean provides high amount of antioxidants . The phenolic acid present in the mungbean seeds is also a kind of antioxidant compound that play crucial role in scavenging radicals and help in converting harmful radicals to less reactive species.

Phenolic compounds in mung bean are secondary metabolite synthesized from the pentose phosphate pathway, the shikimate pathway and the phenyl-propanoid pathway (Randhir *et al.* 2004). These compounds constitute with an aromatic ring with at least one hydroxyl group (Balasundram *et al.* 2006). Phenolic acids are the most common form of phenolic compounds and constituting the most complex groups of phytochemicals in grains (Yao *et al*. 2013). The beneficial effects of phenolic compounds could be due to antioxidant activity (Heim *et al.* 2002). Thus, phenolic compounds can be a major determinant of food antioxidnt potential (Parr & Bolwell. 2000). So they can be a natural source of antioxidants in foods (Balasundram *et al.* 2006)

Majority of the wild *Vigna* accessions demonstrated higher flavonoids (TFS) than released varieties. *V.trilobata* contained maximum TFS followed by *V. umbellata* (>30mg g^{-1}) and lowest was detected in Pusa Vishal (19.65 mg g^{-1}). Flavonoids and phenolic acids both act as antioxidant in mungbeans. Therefore these mungbean genotypes are strong scavengers of

Table 1: Total Flavnoids content (TFC), total phenolic content (TPC), and bound and free phenolic acids in seeds of different mungbean genotypes

			Bound				Free	
Genotype	**TFC**	**TPC**	**Syringic acid**	**Caffeic acid**	**P-coumaric acid**	**Ferulic acid**	**Caffeic acid**	**Ferulic acid**
	(mg g^{-1})			**(µg g^{-1})**			**(µg g^{-1})**	
V sylvestris IC 2770221	25.36	1.86	164.25	3985.62	65.25	141.55	258.63	9.21
MGG 330	28.35	2.05	173.25	3397.82	165.87	136.25	209.65	7.83
V. umbellata IC 251445	30.25	2.98	153.25	3177.5	95.68	135.24	198.75	7.48
TARM 18	24.55	2.3	88.56	2714.5	153.25	131.25	187.65	8.77
V. trilobata I C 349701	32.52	2.38	186.52	1498.65	362.4	132.5	201.32	8.25
Sona mung (landrace)	23.65	2.05	26.85	1625.3	52.36	128.36	215.24	7.12
Pratiksha (Nepal landrace)	24.35	2.31	62.35	451.26	115.26	132.5	198.25	7.45
EC 398889	26.35	2.27	67.58	542.32	88.75	126.58	200.25	7.22
IPM 2-3	21.25	1.98	56.85	562.52	112.5	151.25	187.69	8.21
IPM 205-7	22.38	1.86	72.25	975.25	99.6	140.25	192.5	7.98
IPM 2-14	22.56	1.87	56.55	1912.5	128.65	137.42	188.6	7.65
IPM 409-4	23.88	2.11	62.12	1715.25	92.56	135.2	195.4	7.23
SML 668	23.76	1.95	73.25	1802.62	88.56	133.25	208.55	8.44
Samrat	22.89	1.88	75.25	405.25	112.5	128.93	216.35	7.98
GM 5	23.48	1.78	59.23	625.32	200.56	130.25	210.52	6.95
Pant Mung 5	22.55	1.79	67.45	748.56	95.68	130.65	199.6	8.25
Meha	21.25	2.02	45.2	528.75	89.89	129.85	215.45	8.05
WGG 37	22.8	2.05	55.68	1752.25	118.25	128.47	218.45	7.75
TARM 1	23.54	2.11	72.55	1285.65	72.58	132.58	208.62	7.89
VBG 04-003	22.68	2.15	58.96	2001.23	68.57	133.25	201.45	8.65
Saptari	24.25	1.97	142.52	1567.45	116.87	133.79	196.35	8.23
HUM 16	21.56	1.95	163.99	1625.25	113.56	128.7	189.56	8.56
KM 2241	20.85	1.91	65.98	542.56	75.85	132.55	205.23	7.14
K 851	22.32	2.01	68.45	670.35	127.58	189.25	193.5	6.87
DGGV 2	23.25	2.05	45.82	715.25	122.62	200.65	187.5	7.23
Pusa Vishal	19.65	1.89	49.5	812.36	117.25	215.45	211.3	8.36
CO 5	21.11	1.92	60.47	665.35	98.55	201.65	215.82	6.98
PUSA 9531	23.22	1.97	62.35	1675.32	111.25	132.8	188.59	7.12
ML 818	22.3	2.06	58.3	2065.23	88.65	129.75	210.33	8.45
MH 421	22.19	1.11	73.25	1625.35	101.62	152.52	196.55	6.86
Mean	23.6	2.02	82.29	1455.7	115.55	142.76	203.83	7.84
CD @ 5%	1.05	0.35	26.53	224.52	43.22	5.4	7.52	0.22

superoxide radicals and thus nutritive values of the food as health promoting effects are enhanced..

Flavone, isoflavone, flavonoids, and isoflavonoids are the important metabolites found in the mungbean (Prokudina *et al* 2012., Wang *et al*. 2008). Vitexin (apigenin-8-C-*â*-glucopyranoside) and isovitexin (apigenin-6-C-*â*-glucopyranoside) are present in mungbean seeds at about 51.1 and 51.7 mg g^{-1} respectively (Li *et al.* 2012; Dong et al. 2008). Most flavonoids are classified as polyphenols and have polyhydroxy substitutions with antioxidant activity. Flavonoids play important role in stress protection (i.e., oxidative and temperature stress), early plant development, signaling (i.e., legume nodulation), and protection from insect and mammalian herbivores .

The antioxidant activity in seed extracts all mungbean genotypes are shown in Table 2 using two methods based on radical scavenging capacity by the DPPH (1,1-diphenyl-2-picrylhydrazyl) method and another method, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonate) radical cation (ABTS), reduction by an antioxidant into colourless ABTS, which can be measured spectrophotometrically.

The DPPH method showed a range of antioxidant activity with maximum >30 µmol g^{-1} recorded in three wild accessions *V. sylvestris*, *V. umbellata* and *V.trilobata* and lowest in ML 818 (28.13 µmol g^{-1}). The total antioxidant activities measured by the ABTS method ranged from 3.58 to 15.26 µmol g^{-1}. However, the trend remained almost similar as obtained by DPPH method. The maximum activity was observed in *V. sylvestris* while minimum obtained in DGGV 2 .

The antioxidant activity of 30 mungbeans

Table 2: Antioxidant activities of different mungbean genotypes

Identity	DPPH (μmol g^{-1})	ABTS (μmol g^{-1})
V sylvestris IC 2770221	35.25±1.12	15.26±2.22
MGG 330	32.32±1.18	4.58±2.32
V. umbellata IC 251445	37.82±0.85	11.27±3.11
TARM 18	29.58±0.68	6.57±4.23
V. trilobata I C 349701	35.35±3.12	8.55±1.95
Sona mung (landrace)	30.28±2.71	5.45±1.25
Pratiksha (Nepal landrace)	34.52±2.67	6.15±3.22
EC 398889	31.76±2.24	7.55±2.42
IPM 2-3	30.39±0.65	12.4±1.09
IPM 205-7	35.34±1.48	9.25±3.22
IPM 2-14	32.88±0.65	5.57±3.11
IPM 409-4	28.45±2.10	6.24±1.72
SML 668	30.25±1.28	4.55±1.86
Samrat	30.66±0.25	7.42±1.22
GM 5	31.48±1.23	9.35±2.95
Pant Mung 5	28.96±2.35	8.02±1.63
Meha	29.55±3.22	6.09±1.28
WGG 37	32.58±2.28	6.58±1.33
TARM 1	32.88±0.25	7.05±2.45
VBG 04-003	33.44±2.24	7.52±1.06
Saptari	31.22±1.98	4.25±0.65
HUM 16	32.74±2.21	5.68±0.85
KM 2241	28.65±1.57	6.85±2.60
K 851	32.19±2.58	3.85±1.11
DGGV 2	29.02±0.78	3.58±2.15
Pusa Vishal	32.87±4.27	6.58±2.43
CO 5	28.77±2.05	4.57±0.98
PUSA 9531	30.20±0.45	7.75±1.08
ML 818	28.13±2.24	5.55±1.25
MH 421	32.52±2.07	6.66±2.52

evaluated through DPPH and $ABTS^{+}$ free-radical-scavenging capacity showed as significant positive correlations of $ABTS^{+}$ free-radical-scavenging capacity with total phenolic acids and total flavonoid contents. Correlation coefficients for TPC and TFC with DPPH and ABTS assays are shown in the Table 3.The results showed that TFC and TPC were significantly (P<0.001) correlated with ABTS assay with correlation coefficients of 0.875and 0.708 (Table 3). This suggested that higher the amount of TPC and TFC present in the seed higher will be the antioxidant activity and radical scavenging capacity.These results suggest that these mung bean cultivars are rich in balanced nutrients and that their phytochemicals should be considered as potential sources of natural antioxidants (Shi *et al.* 2016).

Table 3: Correlation of antioxidant activity with TPC, TFC and individual phenolic acids

	DPPH	ABTS
TFC	0.335	0.875**
TPC	0.198	0.708**
Bound syringic acid	-0.098	0.211
Bound caffeic acid	-0.312	0.41
Bound p-coumaric acid	0.214	0.357
Bound ferulic acid	0.265	0.168
Free caffeic acid	0.287	-0.115
Free ferulic acid	-0.214	0.225

Significant at P<0.01(2-tailed test

Antioxidant activity of different seed coat colour genotypes was evaluate to find out the role of colour pigmentation on phenolic compounds. Since green colour mungbeans are abundantly available in this group four genotypes with green seed colour such as ML 818, SML 668, IPM 205-7 and IPM 02-3 were included and compared with dark-green coloured seed coat genotype EC 398889, one yellow seed Sona mung and pale brownish *V.umbellata.* It was observed that dark coloured seed coat (brownish pale and dark green) genotype or yellow seed contained higher antioxidant activity than normal abundantly present green seed coat genotype (**Fig 1**). Mungbeans are rich in phenolic acids which contribute to antioxidant activity. The dull green or pale brownish mung bean wild accession *Vigna umbellata* had the highest value of total flavonoid (TFC) and total phenolic acid (TPC) and also exhibited the highest antioxidant activity followed by yellow sona mung (Fig 1). Among the green colour seed coat shiny group, showed the maximum variation in antioxidant activity. Similar results have been demonstrated in mungbean genotypes having dull green mung bean cultivar of Vima 1 with highest value of total flavonoid and phenolic content (6.58 mg GAE/g) (Yusnawan and Kristiono, 2019). Mungbean is rich in phenolic acids which contribute to antioxidant activity. The total phenolic contents of the dull group varied from 3.97 to 6.58 mg GAE/g, slightly higher than the contents in shiny green group (3.74 to 5.20 mg GAE/g). Therefore, the investigation was undertaken with an objective to establish genetic variation in phenolic acid and flavonoids content in relation to seed coat colour and also to correlate their chemical properties as antioxidants.

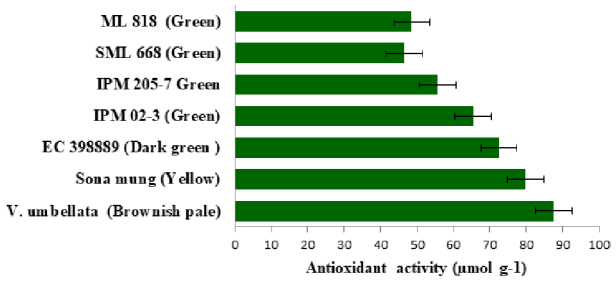

Fig. 1: Antioxidant activity in mungbean having different seed coat colour

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Biochemical studies of chickpea grain, *dal* and fractions of milling by-product

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ABSTRACT

Milling is the process of dehusking and splitting of whole pulse grains to improve the culinary properties. The milling by-product, mixture of husk and cotyledon powder, is rich in bioactive compounds, *viz*., protein, phenol and antioxidants, but often utilized as low value cattle feed. The present study encompasses the biochemical properties of whole seed, *dal* and fractions of chickpea milling by-products for potential edible and therapeutic usage. Chickpea cultivars IPC-11-112 and DCP-92-3 were milled in lab scale grain testing mill. Milling by-product was fractionated with the help of electromagnetic sieve shaker to obtained fractions >1.00, >0.25 and <0.25 mm particle sizes. Biochemical estimation of by-product fractions revealed that for both the varieties, milling by-product fraction >1.00 mm was rich in phenol content and antioxidant activity, whereas fraction <0.25 mm had higher protein content than cotyledons, indicating location of protein globules in peripheral region of cotyledons. Calorific values of the product (*dal*) and by-product were also determined. Chickpea milling by-product was observed to be rich in nutritional and bioactive components, hence, can be utilized for human consumption and health.

***Key words*:** Antioxidant activity, Calorific value, Chickpea cotyledons, Milling by-product, Phenol content, Protein

INTRODUCTION

In a country like India, where large vegetarian population depends upon plant sources for proteins, pulses are essential component of daily human diet. Among pulses, chickpea has an important place, which is grown and consumed globally and has special preference in developing Asian and African countries (Bhagyawant *et al*., 2015). India is the largest producer and consumer of pulses in the world, and produces a wide range of pulses. Among all pulses, chickpea accounts for more than 25% in total area under pulses and contribute 40% to the total pulse production (Yadav *et al*., 2007). Chickpea is essential part of human diet across the world due to high nutritional and bioactive composition. It is the third most cultivated and second most consumed legume in the world. Chickpea grain contain 11.42-16.42% husk (seed coat) (Uttamrao *et al*., 2018) and remaining part is cotyledons and germ. It has high protein digestibility, low glycaemic index, rich in vitamins and minerals and relatively free from anti-nutritional compounds compare to other pulses (Wood and Grusak, 2007). The husk of chickpea is lightly attached to cotyledons due to less gummy substances present in between (Vishwakarma *et al*., 2018) compare to other pulses. So, for complete and easy dehulling of pulses, pre-milling treatments are required to loosen the seed coat. Milling of chickpea yields, dehusked splits (*Dal*), broken and, mixture of husk and cotyledon powder, as by-product. The inner part of the chickpea seed, i.e., cotyledon is rich in protein, carbohydrates and vitamins. Chickpea is tremendous source of protein (18-29%) (Uttamrao, et.al. 2018), carbohydrates (59-65%), lipids (4.5-6.6%), fibres (3-17%), and ash (2.48-3.50%) (Raza *et al*., 2019). The milling by-product, thus obtained, is rich in polyphenolic compounds, antioxidants, dietary fibre, protein, vitamins etc. Chickpea seeds are rich in nutritionally important bioactive components, vitamins and minerals (calcium, iron and phosphorous) (Hirdyani, 2014, Bhagyawant *et al*., 2015 and Geetha *et al*., 2019). Besides many nutritional benefits, chickpea also possesses many anti-nutritive factors, like trypsin and chymotrypsin inhibitors to inhibit protein digestion, á-amylase inhibitor to inhibit starch digestion and also the phytates and oxalates to inhibit mineral absorption. To reduce anti-nutritional factors before consumption various pre-treatments, such as soaking, dehusking, roasting, milling, germination, sprouting, fermentation, boiling, parching, frying and steaming etc. are adopted traditionally. These treatments improve texture, flavour and digestibility of proteins

and carbohydrates. Pre-treatment of legumes also result into slight increase in nutrients, i.e., protein, carbohydrates, soluble dietary fibre (Costa *et al.*, 2006, Saleh *et al.*, 2006, Mittal *et al.* 2012, Bulbula *et al.*, 2018, Dandachy *et al.*, 2019, Olika *et al.*, 2019, Raza *et al.*, 2019). Chickpea seeds are used for medicinal purpose for treatment of bronchitis, leprosy, skin diseases, cancer, cardiovascular disease, type 2 diabetes, digestive diseases (Jukanti *et al.*, 2012, Wallace *et al.*, 2016) and liver infections, and possess anthelmintics properties. It is also used as the cholesterol-lowering food compared to other legumes. Chickpea is considered as a good source of dietary protein because it has good amount of balanced amino acids, high protein bioavailability and relatively low of anti-nutritional factors (Esmat *et al.*, 2010**)**. Chickpea protein also have emulsifying properties and foaming characteristic which can be used for making gluten free bread, bun, cake etc**.** (Aguilar *et al.*, 2015).

Present study was conducted to determine nutritional profile, *viz*., protein content, total phenols, total antioxidant activity and calorific values of whole grain, *dal* and fractions of chickpea milling byproducts so that these fractions can be utilized in preparation of different value-added edible products, which otherwise consumed as low value animal feed. Protein rich powder component of pulse milling by-product can find direct application as substitute to *dal* whereas phenol, antioxidant and fibre rich husk fraction can be used as nutraceuticals and functional food with therapeutic advantages.

MATERIALS AND METHODS

Sample preparation

Whole chickpea grains were cleaned, graded, washed and soaked for 2 hours. The soaked grain was dried up to 8-10% moisture and milled in lab scale universal grain testing mill using abrasive dehusking surface to obtain *dal* and milling by-product. Dehusked splits and broken of cotyledon falls into the central compartment of the universal grain testing mill and by-product was collected in the outer box. The milling by-product was fractionated with the help of electro-magnetic sieve shaker (Electrolab EMS-8) into three fractions, *viz*., >1 mm size, > 0.25 mm size and < 0.25 mm size. All the fractions were represented in percentage form. The samples of dried whole seeds, *dal*, by-product and fractions of by-product were further converted into powder form using laboratory grinder (Perten) for biochemical analysis, including estimation of protein, total phenolic content, total antioxidant activity and calorific value.

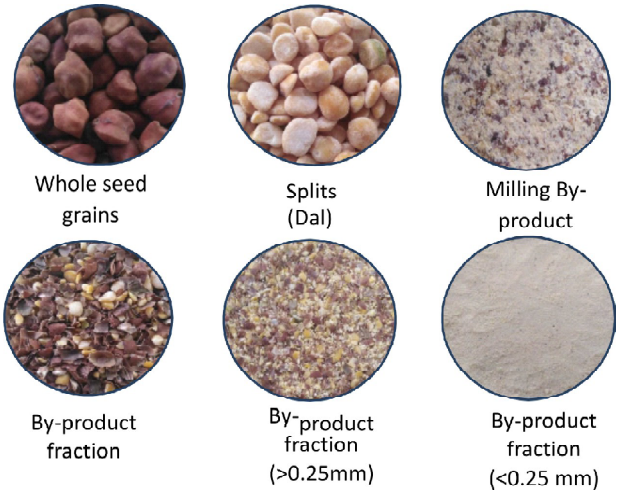

Fig. 1. Whole, *dal* and fractions milling by-product of Chickpea

Estimation of protein content

Protein was extracted from the samples using the slightly modified method of Maehre *et al.*, 2016. 100 mg of powdered sample was grinded in the 10 ml of grinding solution (0.1M NaOH in 3.5 % NaCl). Further, mixture solution was incubated at 60°C in water bath for 90 min. followed by centrifugation at 6000 rpm for 10 minutes. 50µl of supernatant was used for protein quantification using the Lowry's method (Lowry *et al.*, 1951).

Estimation of total phenolic content

Total phenol was extracted and analysed in the sample using spectrophotometric method (Singleton and Lamuela-Raventos 1999). 500 mg of ground sample was mixed in 70% ethanol followed by shaking at 200 rpm for 3 hours in shaker. Then, mixed sample was centrifuged at 6000 rpm for 15 minutes and supernatant was collected. Pellet was re-extracted and supernatant of both were pooled together. 200 µl of supernatant was kept in test tube followed by addition of 250 µL of 1 N Folin-Ciocalteu's reagent, 3 ml of double distilled water and 750 µL Na_2CO_3 (7%) sequentially. The reaction mixture was subjected to vortexing followed by incubation of 8 minutes. After 30 minutes, 800 µL of double distilled water was added and absorbance was measured at 765 nm in spectrophotometer. Phenols in the sample were calculated as gallic acid equivalents (mg of GAE /100 g sample).

Estimation of total antioxidant activity

Total antioxidant in the sample was measured using CUPRAC method (Apak *et al.* 2007). 200 mg of

sample was kept in 20 ml of 70% acetone overnight. Then, it was centrifuged at 6000 rpm for 10 min. 100 µL of supernatant was taken in the test-tube containing 1 ml Neocuproine (2,9-dimethyl-1,10-phenanthroline) alcoholic solution, a copper (II) chloride solution, and 1 ml ammonium acetate aqueous buffer at pH 7 in a test tube. The absorbance was recorded at 450 nm against reagent blank after 30 minutes incubation and antioxidant capacity was expressed as Trolox (6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid) equivalent in terms of m mole TE/100 g sample using following formula.

$$\mu mol\ TE/g = (A_f / \epsilon\ TR)\ (V_f / V_s)\ r\ (V_{initial}/m)$$

where, $V_{initial}$ = initial volume

m = weight of sample

r = dilution factor

V_f = final volume

V_s = volume of aliquot

A_f = absorbance

$\epsilon\ TR = 1.67 \times 10\ 4\ L mol^{-1}\ cm^{-1}$

Estimation of calorific value

The calorific value of each sample of chickpea was determined with the help of IKA C200 Bomb Calorimeter. The readings were calibrated as per benzoic acid tablets. For isoperibol testing water temperature was maintained at 20-25^{0}C. Bomb is placed in calorimeter after filling oxygen at 30 psi. Observations was recorded in Cal/g and expressed in kcal/100 g. Procedure recommended by the manufacturer of the calorimeter was adopted.

All the observations were recorded in triplicates and the data presented with average mean values (± S.D.). Significance test was accepted at $p \leq 0.05$.

RESULTS AND DISCUSSION

Dal and by-product recovery

The *dal*, obtained from the selected cultivars, namely, IPC-11-112 and DCP-92-3, were observed to be 65.47 and 74.07% respectively, whereas the 34.53 and 25.93% milling by-product were generated respectively. The fractionation of by-product, yielded 65.03 and 75.31% of husk and broken rich by-product fraction retained above 0.25 mm sieve. Remaining 36.40 and 23.39%, powder fraction, passes through the 0.25 mm sieve for the two cultivars, respectively. This indicates that about 30% powder component can be extracted from the milling by-product obtained from abrasive dehusking method of chickpea. This fraction is similar to chickpea cotyledon powder (*Besan*) and can be utilized for edible purposes.

Protein content

The protein content of whole seeds of the selected cultivars, *viz.*, IPC-11-112 and DCP-92-2 were observed to be 17.76 and 18.83%, respectively, whereas that of dehusked splits (*dal*) were found to be 18.33 and 22.36% for the two cultivars, respectively. The average value of protein content for whole grains and splits of the evaluated genotypes were observed to be 18.30 and 20.35%, respectively. Overall protein content of milling by-product obtained from the two cultivars had the average value of 10.56%. By-product fraction >1.00 mm, had the lowest protein values of 9.83 and 8.71% for the selected cultivars. The fraction between 1.00 mm and >0.25 mm had the average protein content of 12.75%. The highest protein content of 20.86% and 23.48% were reported in both varieties IPC-11-112 and DCP-92-3, respectively, for the by-product fraction <0.25mm sieve size. The by-product fraction >1.00 mm had the lowest protein (9.27%) due to high amount of husk and fiber. The middle fraction (>1 to >0.25) reported to have average protein content of 12.75% due to presence of the broken of cotyledons. The powder component, <0.25mm particle size, had the highest protein content of 22.17%, which is even more than the dehusked split (20.35%) indicating location of protein molecules in the peripheral region (aleurone layer) of cotyledons. Thus, this component (<0.25mm) of milling by-product can be utilized as source for pulse protein in making protein rich edible products. Protein contents of *dal* and powder component of milling by-product were compared using t-test and protein content in powder was observed to be significantly higher in by-product powder at $p \leq 0.05$.

Total phenolic content

The total phenolic content (TPC) of whole grain, *dal*, by-product and fractions of milling by-product for the selected two varieties of chickpea were estimated. TPC for the whole grains were observed to be 155.28 and 97.31 mg GAE/100 g for the varieties IPC-11-112 and DCP-92-3, respectively, with an average value of 126.29 mg GAE/100 g. *Dal* obtained after dehusking had shown the lowest TPC of 127.54 and 59.00 mg GAE/100 g for the two cultivars respectively. This reduction can mainly be attributed to absence of husk on *dal*. By-product of the two cultivars had the TPC of 214.49 and 161.90 mg GAE/100 g respectively. The husk fraction between <1.00 mm and >0.25mm contains the highest phenolic contents, *viz.*, 212.84 mg GAE/100g in IPC-11-112 and 226.09 mg GAE/100 g

in DCP-92-3. Average value of the fraction was observed to be 219.47 mg GAE/100 g. The powder fraction (<0.25mm) had the higher TPC than the *dal* indicating presence of fine husk in the powder fraction. TPC of the fraction were observed to be 151.14 and 82.40 mg GAE/100 g, with an average value of 116.77 mg GAE/100 g. Total phenolic content of whole grain and milling by-product fraction >0.25 mm was compared using t-test and the difference was highly significant at $p \leq 0.05$.

Total antioxidant capacity

The antioxidant value for the whole seeds of chickpea cultivar IPC-11-112 and DCP-92-3 was observed to be 5.84 and 4.84 mmole TE/100g, respectively. Dehusking of grains showed the reduction in antioxidant value of *dal*, which were observed to be 1.89 and 2.23 mmole TE/100 g for the selected varieties respectively. Reduction in average antioxidant value from whole grain to *dal* was observed mainly due to removal of husk. It reduced from 5.34 mmole TE/100 g in whole chickpea grain to 2.06 mmole TE/100 g for *dal*. For the milling by-product of the two cultivars, antioxidant values were 27.67 and 21.63 mmole TE/100 g, respectively, with an average value of 24.65 mmole TE/100 g for the cultivars. By-product fraction >1.00 mm was observed to have the highest antioxidant values of 50.71 and 44.94 mmole TE/100 g for the two cultivars respectively, which was higher than the fractions <1.00 mm. This indicates that the maximum husk fraction is retained by the 1.00 mm sieve. The average antioxidant value for by-product fraction >1.00 mm was observed to be 47.82 mmole TE/100 g. By-product fraction <1.00 and >0.25 mm, had been recorded average antioxidant value of 29.91 mmole TE/100 g. For the fraction <0.25 mm, the values were observed to be 3.21 and 3.89 mmole TE/100 g for the two cultivars respectively, with an average value of 3.55 mmole TE/100 g. Though the highest antioxidant activity was observed in husk fraction >1mm sieve size but for therapeutic usage both the fractions, i.e, >1.00 and >0.25 mm sieve can be utilized higher antioxidant value with 12.75% protein in the mid fraction. The difference between antioxidant activity of milling by-product fraction >0.25 mm was observed to be significantly higher in comparison to whole grain at $p \leq 0.05$. Marathe *et al.*, 2011 and Xu and Chang, 2007 found the direct correlation between the phenolic content and antioxidant activity of the edible products. Presence of polyphenols in husk has the capacity of scavenging of free radicals, thus, exhibiting antioxidant activity preventing damage to bio-molecules like 2-deoxy D-ribose and hemoglobin (Tiwari and Singh, 2012).

Calorific value

Calorific value was determined by using bomb calorimeter (IKA Make). The amount of energy observed to be 408.90, 407.97, 395.86, 380.12, 392.52 and 369.66 kcal/100gm for whole seed, *dal* (cotyledons), milling by-product, and fractions, >1mm, <1.00 to >0.25mm and <0.25mm sieve sizes respectively, for cultivar IPC-11-112. For the cultivar, DCP-92-3, the calorific values were observed to be 391.30, 395.72, 376.16, 360.32, 368.42 and 398.28 kcal/100gm for the whole seed, *dal*, by-product and different fractions, respectively. The average calorific values of chickpea whole, *dal*, by-product and its fractions varied between 370.22 to 401.55 kcal/100g. Calorific values of the whole, *dal* and milling byproducts were compared using t-test, which indicates no significant difference at critical value at $p \leq 0.05$.

Table 1. Biochemical Components of Chickpea (IPC-11-112 and DCP-92-3)

Chickpea	Protein Content (%)	Antioxidant Activity (m mol TE/100 g)	Phenol Conc. (mg GAE/100 g)	Calorific Value (kcal/100 g)	Recovery (%)
i) IPC-11-112					
Whole seed	17.76 (0.82)	5.84 (0.28)	155.28 (5.93)	408.90	-
Dal	18.33 (0.51)	1.89 (0.05)	127.54 (1.29)	407.97	59.13
By-product	6.17 (0.10)	27.67 (5.03)	214.49 (11.13)	395.86	34.53
>1.00 mm	9.83 (0.05)	50.71 (4.07)	184.27 (3.13)	380.12	35.21
> 0.25 mm	14.60 (0.40)	27.10 (0.82)	212.84 (9.16)	392.52	26.76
<0.25mm	20.86 (0.40)	3.21 (0.10)	151.14 (3.80)	369.66	38.03
ii)DCP-92-3					
Whole seed	18.83 (1.53)	4.84 (0.19)	97.31 (2.35)	391.30	-
Dal	22.36 (0.45)	2.23 (0.05)	59.01 (3.29)	395.12	69.86
By-product	14.95 (0.10)	21.63 (1.39)	161.90 (1.90)	376.16	25.93
>1.00 mm	8.71 (0.20)	44.94 (2.28)	183.44 (5.02)	360.32	47.66
> 0.25 mm	10.90 (0.15)	32.73 (1.27)	226.09 (8.15)	368.42	28.65
<0.25mm	23.48 (0.45)	3.89 (0.08)	82.40 (0.36)	398.28	23.70

Table 2. Average value of Biochemical components of Chickpea

Chickpea	Protein Content (%)	Antioxidant Activity (m mol TE/100 g)	Phenol Conc. (mg GAE/100 g)	Calorific Value (kcal/100 g)	Recovery (%)
Whole seed	18.30	5.34	126.29	400.10	-
Dal	20.35	2.06	93.27	401.55	64.50
By-product	10.56	24.65	188.20	386.01	30.23
>1.00 mm	9.27	47.82	183.85	370.22	41.43
> 0.25 mm	12.75	29.91	219.46	380.47	27.70
<0.25mm	22.17	3.55	116.77	383.97	30.86

The observed value for protein content was within the range of 18-29% for chickpea reported by Raza *et al.*, 2019. Total phenols and total antioxidant activity of whole seeds lies within the reported range, i.e., 38.6-542.7 mg GAE/100g and 3.5-11.08 mmole TE/ 100 g, respectively (Parikh *et al.*, 2018), which is similar to observed values reported in this study.

The observations of biochemical components of chickpea cultivars and the average values are compiled and presented in Table-1 and Table-2, respectively. Different biochemical parameters and recovery data have been given in graphical representation and presented in figures 2-6.

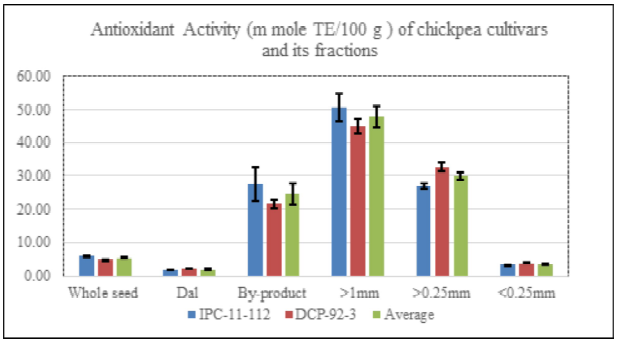

Fig. 4. Antioxidant activity (m mol TE/100 g) of chickpea cultivars and its fractions

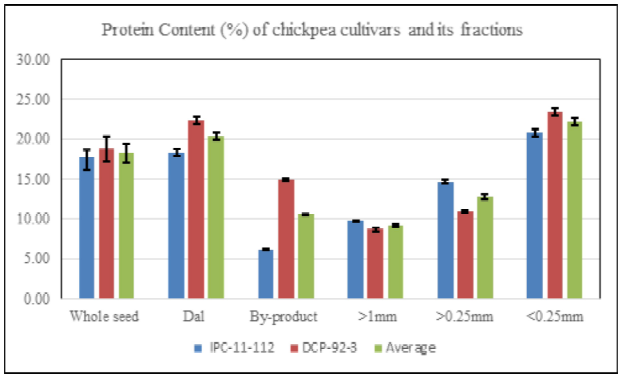

Fig. 2. Protein Content (%) of chickpea cultivars and its fractions

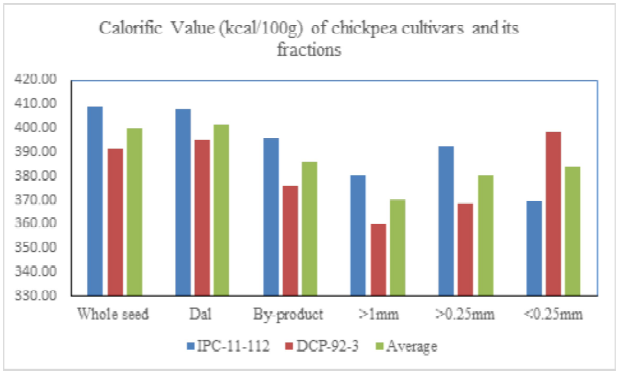

Fig. 5. Calorific Value (kcal/100g) of chickpea cultivars and its fractions

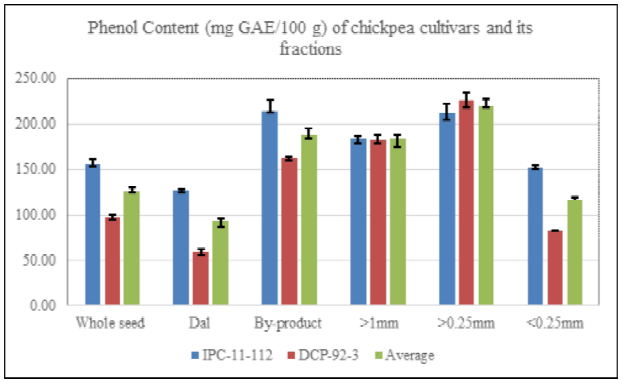

Fig. 3. Phenol Content. (mg GAE/100 g) of chickpea cultivars and its fractions

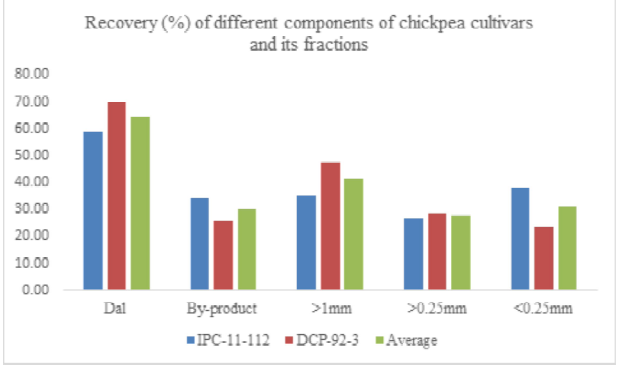

Fig. 6. Recovery (%) of chickpea cultivars and its fractions

CONCLUSION

The biochemical evaluation of fractions of chickpea milling by-products shows that fraction >1mm size, mainly husk, is rich in antioxidant (47.82 m mol TE/100 g) and phenol 183.85 (mg GAE/100 g), therefore, this fibre rich fraction can find application in development of functional foods and nutraceutical products. Milling by-product fraction less than <0.25mm size have protein content (22.17%) which is higher than that of dehusked cotyledons, therefore, can be utilized as substitute to *dal* and used directly in development of traditional home recipes and protein rich commercial products, *viz*., wafers, nachos, protein shake, protein soup etc. The by-product as such can be used in making bakery products rich in fiber and proteins. The fractions of chickpea milling by-product also have therapeutic properties, thus, can be used as nutraceutical, antioxidants, cholesterol lowering fibers and anti-cancerous edible product. Development of food products from such a low-cost source milling by-product and fractions of chickpea can be a useful alternative strategy to combat malnutrition and increasing availability of pulse proteins for vegetarian population.

ACKNOWLEDGEMENTS

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JFL

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Short Communication

Co-heritability and correlation of yield and associated characters in winged bean (*Psophocarpus tetragonolobus* L.DC.)

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ABSTRACT

Estimation of co-heritability and correlation between yield and its associating characters was evaluated in twenty three genotypes of winged bean (*Psophocarpus tetragonolobus* L.DC.). The results from co-heritability studies revelaed that character pairs viz., days to 50% flowering with pod length, dry pod weight, pod yield/plant, protein content and seed yield/plant indicates the joint selection for these characters. Correlation coefficient studies reported that seed yield/plant had the highest estimates of positive correlation and significance, both at genotypic and phenotypic level, with days to 50% flowering (0.408, 0.344), days to maturity (0.330, 0.285), secondary branches/plant (0.321, 0.350), biological yield/plant (0.453, 0.400), 100 seed weight (0.333, 0.294), dry pod weight (0.300, 0.266) and pod yield/plant (0.377, 0.355). These characters , thus, would be advantageous for increasing yield in winged bean.

Key words: Co-heritability, Correlation, Genotypes, Winged bean, Yield.

Winged bean is a leguminous crop with nutritional content and medicinal value grown in tribal areas of Chhattisgarh. Exact statistics on area and production in Chhattisgarh is still unknown. It is estimated to achieve green pod yield of 124-130 q/ha as well as seed yield 18-20 q/ha in Chhattisgarh (Yadav, 2015). Improvement of yield requires co-heritability and interdependence of quantitative characters with yield of the genetic material. Co-heritability ascribed to the co-inheritance of different character pairs and indicates the genetic progress which could result from the joint selection for these characters. It is a better genetic parameter than genetic correlation, as correlation does not take account for environmental variance which is also a component of phenotypic variance in which selection is applied. Hence, an attempt was undertaken to understand the inheritance of yield and its associating characters and their interdependence in winged bean.

Twenty three genotypes viz., RWB-11, RWB 11-1, RWB 12, RWB 13, RWB14, RWB 15, RWB16, RWB 17, RWB18, RWB 19, RWB-20, RWB 21, RWB 22, AWB 16-2, PWB 11-2, PWB17-1, PWB 17-9, AWB 18-2, AKWB-1(C), IWB 1, RMD WB 1 and C.G. Chaudhari sem-2(C) of winged bean were obtained from All India Coordinating Centres of BAU, Ranchi, Rahuri, Akola, Ambikapur and IGKV, Raipur. The experiment was carried out in randomised block design with three replications at research farm, IGKV, Raipur during *Kharif* 2018. All the cultural operations were followed uniformly for all the genotypes under study. Each plot consisted of 3 m length with row to row and plant to plant distance of 60 cm and 30 cm, respectively. Observations on 15 characters (Table-1) were taken on five competitive plants from each replication. Co-heritability was calculated by Singh and Chaudhary (1985) and correlation coefficient was estimated as per the formula suggested by Miller *et al*. (1958).

The co-heritability estimates of character combinations are presented in Table-1. High and positive co-heritability values were observed for the character pairs viz., days to 50 % flowering with pod width, dry pod weight, pod yield/plant, protein content and seed yield/plant; days to maturity with biological yield/plant, 100 seed weight, harvest index, pod yield/plant and seed yield/plant; plant height with seeds/pod, biological yield/plant and dry pod weight; pod length with seeds/pod, biological yield/plant, pod yield/plant and seed yield/plant; pod width with seeds/pod, dry pod weight and seed yield/plant; secondary branches/plant with seed/pod and dry pod weight; pods/plant with harvest index, protein content and seed yield/plant; seeds/pod with biological yield/plant and dry pod weight; biological yield/plant and 100 seed weight with seed yield/plant; harvest index with dry pod weight; dry pod weight with protein content and seed yield/plant. Similar findings was also reported by Yadav (1996) for 100 seed weight with seed yield/plant in chickpea, Yadav (2007) for days to maturity, 100 seed weight

and protein content with seed yield/plant in soybean and Yadav (1992) for branches/ plant, 100 seed weight with seed yield/plant in chickpea. This suggests that selection for either of these attributes may result in simultaneously selection for other co-herited characters. Smaller magnitude of co-heritability estimates are expected due to high magnitude of environmental variance, hence resulting poor response to selection.

The magnitude and nature of the correlation of characters at genotypic and phenotypic levels are presented in Table-2. In general, the estimates of genotypic correlation were higher than the phenotypic correlation, indicating a strong inherent association between the traits. Genotypic and phenotypic correlation revealed that seed yield/plant was significantly positive correlation with days to 50 % flowering, days to maturity, secondary branches/ plant, biological yield/plant, 100 seed weight, dry pod weight and pod yield/plant. Similar findings were reported by Pandita *et al*. (1989) for plant height with pod length, Monitor *et al*. (1997) for seed yield with 100 seed weight and biological yield/plant, Nandan *et al*. (2009) for seed yield/plant with 100 seed weight and dry pod weight, Yadav (2018) for seed yield with pod yield and days to flowering, and Mohamad and Madalgeri (2012) for seed yield with dry pod weight respectively. Seed yield had negative and significant correlation with protein content. Similar finding was observed by Mohamad and Madalgeri (2012) for seed yield with protein content in winged bean.

Significant and positive associations were observed for days to 50% flowering with pod length (0.391, 0.381), pods/plant (0.467, 0.462) and biological yield/plant (0.803, 0.455); for days to maturity with 100 seed weight (0.622, 0.464) and harvest index (0.342, 0.276); for plant height with pod length (0.431, 0.404) and dry pod weight (0.776, 0.579); for Pod length with pod width (0.595, 0.609) and seeds/pod (0.383, 0.309); for pod width with pods/plant (0.318, 0.317), seeds/pod (0.354, 0.257) and dry pod weight (0.564,0.511); for secondary branches/plant with pods/plant (0.305, 0.296) and dry weight (0.375, 0.339); for pods/plant with 100 seed weight (0.397, 0.382) and for seeds/pod with pod yield/plant (0.310, 0.303), respectively at genotypic and phenotypic levels.

Significant but negative correlations were showed for days to 50% flowering with secondary branches/ plant (-0.512, -0.505); for days to maturity with pods/ plant (-0.570, -0.561); for plant height with pod yield/ plant (-0.460, -0.446); for pod length with dry pod weight (-0.324, -0.283), protein content (-0.394, -0.338); for pod width with biological yield/plant (-0.346,-0.272); for secondary branches/plant with pod yield/ plant (-0.396, -0.394); for 100 seed weight with protein content (-0.477, -0.433); and pod yield/plant with protein content (-0.304, -0.285) respectively at both genotypic and phenotypic levels. Similar findings were also reported by Mohamad and Madalgeri (2012)

Table 1. Estimation of co-heritability for different paired characters in winged bean.

Characters	Days to flowering	Days to maturity	Plant height	Pod length	Pod width	Secondary branches/ plant	Pods/ plant	Seeds / pod	Biological yield/ plant	100 seed weight	Harvest Index	Dry pod weight	Pod yield/ plant	Protein content	Seed yield/ plant
Days to flowering	-	1.04	1.00	1.02	1.10	– 1.01	1.01	– 1.25	– 1.8	–1.36	1.03	1.22	1.7	1.19	1.18
Days to maturity		-	– 0.98	– 1.02	–1.00	– 1.02	–1.01	– 1.29	1.28	1.34	1.23	0.98	2.0	–1.47	1.15
Plant height			-	1.06	– 0.97	– 1.03	1.00	1.54	1.23	1.02	– 1.45	1.34	– 1.03	–1.10	– 0.72
Pod length				-	0.97	– 1.09	– 1.00	1.23	4.85	–1.14	1.05	–1.14	1.45	–1.16	1.11
Pod width					-	–0.96	1.00	1.37	– 1.27	–1.45	– 1.10	1.10	–1.18	1.06	1.24
Secondary branches						-	1.03	1.27	– 1.28	1.06	0.96	1.10	– 1.00	– 1.71	0.91
Pods/plant							-	0.36	–1.28	1.03	1.47	–1.16	1.01	1.12	8.70
Seeds/pod								-	1.54	1.02	1.03	1.14	1.02	1.07	–1.21
Biological yield/plant									-	1.00	1.09	– 1.28	1.00	–1.09	1.13
100 seed weight										-	1.01	–1.10	– 1.04	– 1.10	1.13
Harvest index											-	1.13	–1.00	– 1.05	– 0.85
Dry pod Weight												-	1.05	1.21	1.12
Pod yield/plant													-	– 1.06	1.06
Protein content														-	– 1.13

Table 2. Genotypic and phenotypic correlation coefficient matrix among different characters of winged bean.

Characters	Level	Days to flowering	Days to maturity	Plant height	Pod length	Pod width	Secondary branches/ plant	Pods/ plant	Seeds / pod	Biological yield/ Plant	100 seed weight	Harvest Index	Dry pod weight	Pod yield/ plant	Protein content	Seed yield/ plant
Days to	G		0.161	0.100	0.391**	0.219	-0.512**	0.467**	- 0.289*	0.803**	- 0.263	- 0.193	0.290	0.053	0.218	0.408**
flowering	P		0.154	0.100	0.381**	0.199	-0.505**	0.462**	- 0.230	0.445**	- 0.193	- 0.186	0.237	0.031	0..182	0.344**
Days to	G			0.113	- 0.214	- 0.149	- 0.037	- 0.570**	- 0.200	0.240	0.622**	0.342**	0.127	0.002	- 0.075	0.330**
maturity	P			0.115	- 0.209	- 0.148	- 0.036	- 0.561**	- 0.154	0.187	0.464**	0.276**	0.129	0.001	- 0.051	0.285**
Plant height	G				0.431**	- 0.082	- 0.254	0.138	0.159	0.174	0.182	- 0.252	0.776**	- 0.460**	- 0.209	- 0.013
	P				0.404**	- 0.084	- 0.245	0.138	0.103	0.141	0.177	- 0.173	0.579**	- 0.446**	- 0.189	- 0.018
Pod length	G					0.595**	- 0.012	- 0.015	0.383*	0.068	- 0.024	0.038	- 0.324*	0.242	- 0.394*	0.087
	P					0.609**	- 0.011	- 0.015	0.309*	0.014	- 0.021	0.036	- 0.283*	0.166	- 0.338*	0.078
Pod width	G						- 0.140	0.318*	0.354**	-. 0346*	- 0.045	- 0.196	0.564**	- 0.245	0.257	0.122
	P						- 0.145	0.317*	0.257*	- 0.272*	- 0.031	- 0.177	0.511**	- 0.207	0.242	0.098
Secondary	G							0.305*	0.202	- 0.214	0.226	0.090	0.375*	- 0.396**	- 0.409**	0.321**
branches	P							0.296*	0.159	- 0.167	0.213	0.093	0.339*	- 0.394**	- 0.239	0.350 **
Pods/ plant	G								- 0.007	- 0.161	0.397**	0.053	- 0.035	0.194	0.096	0.061
	P								- 0.019	- 0.125	0.382**	0.036	- 0.030	0.191	0.085	0.007
Seeds/ pod	G									0.297	0.187	0.231	0.180	0.310*	0.247	- 0.120
	P									0.192	0.182	0.223	0.157	0.303*	0.230	- 0.099
Biological	G										0.228	0.034	- 0.160	0.136	- 0.327*	0.453**
yield/ plant	P										0.226	0.031	- 0.125	0.135	- 0.298*	0.400**
100 seed	G											0.181	0.151	- 0.121	- 0.477**	0.333*
weight	P											0.178	0.137	- 0.116	- 0.433**	0.294*
Harvest index	G												0.556**	- 0.599**	- 0.247	- 0.076
	P												0.492**	- 0.595**	- 0.235	- 0.089
Dry pod	G													- 0.020	0.017	0.300*
Weight	P													- 0.019	0.014	0.266*
Pod yield/	G														- 0.304*	0.377*
plant	P														- 0.285*	0.355*
Protein	G															- 0.426**
content	P															- 0.376**

** Significant at 1% level, * Significant at 5% level

and Kushwaha and Singh (2013) between 100 seed weight and seed yield/plant in winged bean. The fluctuation in association between different characters may be attributed impact of environmental factors on the associates in winged bean genotypes.

It is concluded that selection for high yield in winged bean may be effective by simultaneous improvement of yield associating characters viz., days to 50% flowering, days to maturity, pod length, secondary branches/plant, dry pod weight, biological yield/plant, 100 seed weight and protein content.

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Short Communication

Response of mungbean varieties to sowing time and spacing during summer season

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ABSTRACT

A field experiment was conducted at Instructional Farm of Bidhan Chandra Krishi Viswavidyalaya, Jaguli, West Bengal, India to find out the effect of three sowing dates (15 February, 11 March, 4 April), two row spacings (30 cm, 25 cm) and two varieties (Samrat, Pant Mung 5) on phenological development and growth of mungbean during summer, 2019. Mean time required from sowing to emergence, flower initiation, pod initiation, end of flowering, end of pod formation and maturity were 6.1, 33.5, 41.7, 50.8, 59.9 and 72.8 days, respectively. The duration of mungbean was shortened by 6.7 days with delay in sowing from 15 February (76.0 days) to 4 April (69.3 days). Sowings in mid-March and early April resulted in better vegetative growth in terms of plant height, branching habit and dry matter production due to high temperature compared to early sowing in mid-Fabruary. Mungbean sown on 11 March recorded the highest grain yield (684.3 kg/ha), which was 14.5% and 68.8% greater over 15 February (597.8 kg/ha) and 4 April (405.5 kg/ha) sowings. Close row spacing (25 cm) resulted in greater grain (583.0 kg/ha) and straw yield (2415.7 kg/ha) than wider spaced crop (30 cm). 'Pant Mung 5' accumulated greater total GDD (1214°C day), HTU (9443°C day hour), PTU (15308°C day hour) for entire life cycle, and produced higher grain yield (684.3 kg/ha) over 'Samrat'.

***Key words*:** Mungbean, Phenology, Sowing date, Spacing, Thermal indices, Variety

Mungbean [*Vigna radiata* (L.) Wilczek] is primarily a rainy season crop, but it becomes suitable as a summer crop under irrigated conditions. Summer mungbean is usually sown from the late February to first week of April after the harvest of rapeseed and mustard, lentil, potato etc. in West Bengal. Delayed sown crop usually experience high summer temperature during vegetative phase and norwester shower during reproductive and pod maturation stages, which lead to chaffy pods and yield loss. Thus, optimization of sowing time is important to get optimum phenophase duration, near-synchronous maturity and higher productivity of mungbean. In the context, heat unit concept is a popular agro-meteological component of crop weather models to predict the growth and development process of greengram.

Mungbean is mainly sown by broadcasting in rainfed areas while under irrigated condition mainly line-sown. The growth and yield of mungbean is largely influenced by planting density, so cultivar-specific row spacing needs to be standardized. The commonly cultivated varieties in West Bengal are Sonali (B 1), Panna (B 150), PDM 54, Samrat, etc. (Government of West Bengal, 2012). Besides, some high-yielding, photo-insensitive varieties of mungbean have been developed and released in last two decades in the country, which need to be tested for their adoption under varied seasons in different agro-climatic situations. Thus, evaluation-*cum*-selection of promising mungbean varieties along with optimization of their sowing time and spacing is the priority of present-day research in the state. Keeping these in view, a comprehensive study was done on the effect of sowing dates and spacings on phenology, thermal indices and growth of summer mungbean varieties in new alluvial zone of West Bengal.

A field experiment was conducted for mungbean crop during pre-*kharif* season (summer) of 2019 on a medium land loamy soil at Instructional Farm (22°93' N latitude, 88°53' E longitude and 9.75 m above mean sea level) of Bidhan Chandra Krishi Visvidyalaya (BCKV), Jaguli, Nadia, West Bengal, India. Treatments replicated thrice were assigned in Split-Split-Plot design with three sowing dates (15 February, 11 March and 4 April) in main plots, two row spacings (30 cm and 25 cm) in sub-plots and two varieties ('Samrat' and 'Pant Mung 5') in sub-sub plots.

Soaked seeds @ 30 kg/ha of two mungbean varieties were mixed with *Rhizobium* culture following standard method and then sown in lines at 30 cm and 25 cm apart in 3 m × 3 m experimental plots as per sowing time schedule. The standard crop management practices like uniform fertilizer dose of 20:40:40 kg/ha of N: P_2O_5:K_2O, one hand weeding at 30-40 days after sowing (DAS) and one irrigation at 30-40 DAS were adopted. Two pickings followed by whole-plant harvesting were done during first week of May to second week of June.

The phenophasic development (*viz*. emergence, flower initiation, pod initiation, end of flowering, end of pod formation and maturity) of mungbean varieties at different sowing dates were noted by field inspection at 2-3 days interval. The daily meteorological data were collected from the Department of Agro-meteorology and Physics, BCKV, Mohanpur, West Bengal. The total summed growing degree days (GDD) [GDD= (T_{max} + T_{min}) / 2 – T_b] for entire life cycle was determined by taking a base temperature of 10°C; while heliothermal units (HTU) [HTU= GDD × Bright sunshine hour] and photothermal units (PTU) [GDD × Day length] were calculated by the standard equations.

The plant height, growth attributes like branching habit, nodulation, dry matter (DM) production, crop growth rate (CGR) and grain yield of mungbean were recorded as per standard methods. The recorded data were analysed using Fisher's Analysis of Variance technique following the procedures described by Gomez and Gomez (1984), and the mean differences were compared at 5% level of significance.

Phenological development

Mungbean had indeterminate type of growth habit comprising vegetative and reproductive stage intermingled together for a specific period. Seedlings of two mungbean varieties emerged faster (3.9 days, 4.6 days) in 4 April and 11 March sown plots, respectively compared to the earliest sowing on 15 February (10.0 days) in the study (Table 1). The early-April and mid-March sown crop received 42.2 mm and 7.3 mm rainfall during pre-emergence period, which hastened the germination of seeds as well as emergence in the field. The phenophase-wise average duration of mungbean was: 6.1 days (sowing to emergence), 27.4 days (emergence to flower initiation), 8.2 days (flower initiation to pod initiation), 9.1 days (pod initiation to end of flowering), 9.1 days (end of flowering to end of pod formation), and 12.9 days (end of pod formation to maturity). Mean time required from sowing to emergence, flower initiation, pod initiation, end of flowering, end of pod formation and maturity were 6.1, 33.5, 41.7, 50.8, 59.9 and 72.8 days, respectively. Mungbean sown on 15 February took 76.0 days from sowing to maturity, which was shortened by 3.2 days in 11 March, 6.7 days in 4 April sowings in the study. Similarly, Kumar *et al*. (2020) reported that the duration of greengram was reduced successively with delay in sowing from 1 February (77.5 days) to 15 March (71.7 days). Row spacing could not influence the phenological development of mungbean crop; while 'Pant Mung 5' had slightly longer duration (73.4 days *vs.* 72.1 days) than Samrat. Sowing date × variety could influence significantly the length of two phenophases *viz*. sowing to emergence, and flower initiation to pod initiation in the experiment (data not shown).

Thermal indices

Sowing time caused significant variation in accumulated GDD at five phenophases and life cycle exclusively flower initiation to pod initiation in the investigation (Table 1). The summed GDD for entire life cycle of mungbean was gradually increased with delay in sowing from 15 February (1092°C day) to 4 April (1252°C day). Although there was reduction in number of days due to delayed sowings, but increase in accumulated GDD was noted due to rising of temperature for delay in sowing from mid-February to early April in lower *gangetic* plains of West Bengal. Row spacing had no significant influence on accumulated GDD for life cycle of mungbean.

Mungbean sown on 11 March recorded the highest summed total HTU (9974°C day hour) being at par with late sowing on 4 April (9791°C day hour), but significantly greater over early sowing on 15 February (8187 °C day hour) in the investigation. However, Tijare *et al.* (2017) reported greater summed HTU (12109°C day hour) for late-sown mungbean (30 March) compared to earlier sowings on 1, 10 and 20 March at Akola, Maharastra. Temperature generally governed the onset of different phenophases in mungbean crop, but day length also had influence on photo-thermal requirements of the crop. Although late sowings (11 March and 4 April) of mungbean reduced the duration of the crop, but those accumulated greater PTU due to higher temperature and day length compared to early sowing (15 February). There was vernal equinox (21 March) having equal length of day and night (about 12 hour each) within the experimental period; and after which the day length was slowly increased. So, the crop sown on 4 April experienced only longer day lengths throughout its life cycle

Table 1. Effect of sowing date, row spacing and variety on phenophase duration and thermal indices of summer mungbean

Treatment	Phenolophase duration (days)							GDD (°C day)	HTU (°C day hour)	PTU (°C day hour)
	Sowing to Emergence	Emergence to Flower initiation	Flower initiation to Pod initiation	Pod initiation to End of flowering	End of flowering to End of pod initiation	End of pod initiation to Maturity	Duration (days)			
Sowing date										
15 February	10.0	25.4	10.3	7.9	10.8	11.7	76.0	1092	8187	13283
11 March	4.6	28.7	7.0	9.6	7.7	16.0	72.8	1252	9974	15770
4 April	3.9	28.2	7.6	9.8	9.0	10.9	69.3	1252	9791	16245
CD (P=0.05)	0.38	0.53	1.54	1.78	1.29	1.14	2.10	39.4	309.7	520.7
Row spacing										
30 cm	5.7	27.8	8.0	9.5	9.1	12.6	72.8	1196	9305	15062
25 cm	6.6	27.0	8.6	8.7	9.2	13.1	72.7	1201	9329	15137
CD (P=0.05)	1.0	0.95	1.11	0.85	0.69	0.60	1.01	17.0	139.7	221.1
Variety										
'Samrat'	6.3	27.2	7.7	9.3	9.1	12.5	72.1	1183	9191	14892
'Pant Mung 5'	6.0	27.6	8.8	8.9	9.2	13.2	73.4	1214	9443	15308
CD (P=0.05)	0.36	0.75	0.46	0.82	0.83	0.54	0.68	13.2	128.4	174.2

compared to earlier two sowings (15 February and 11 March). Early April sown mungbean accumulated maximum total PTU (16245 °C day hour), which was 2962 °C day hour and 474°C day hour greater over sowings on 15 February and 11 March, respectively.

'Pant Mung 5' accumulated slightly greater summed GDD (1214°C day), HTU (9443°C day hour) and PTU (15308°C day hour) from sowing to maturity than 'Samrat' (1183°C day, 9191°C day hour and 14892 °C day hour) in the study. This might be due to more days required by 'Pant Mung 5' during four phenophases viz. emergence to flower initiation, flower initiation to pod initiation, end of flowering to end of pod formation and end of pod formation to maturity.

Growth attributes

Delayed sowing on 4 April resulted in maximum plant height (55.6 cm) compared to earlier sowings on 15 February (44.6 cm) and 11 March (54.7 cm) in the experiment (Table 2). This might be due to the fact that rising temperature during March-April had favourable influence on vegetative growth of mungbean plants during summer at Jaguli, Nadia. 'Pant Mung 5' produced significantly taller plants (52.8 cm) compared to 'Samrat' (49.4 cm). The number of branches/plant was gradually increased due to delay in sowing from 15 February to 4 April, but no significant variation in branching habit was noted between two spacings and two varieties tested in the study. Gebremariam and Baraki (2018) found significant variation in number of branches/plant of mungbean due to five inter-row spacings (20 cm, 25 cm, 30 cm, 35 cm and 40 cm) but non-significant difference among intra-row spacings (5cm, 10 cm, 15 cm and 20 cm) at Humera, Ethiopia. But Kalsaria *et al.* (2017) reported greater number of branches/plant of greengram plant spaced at 45 cm × 10 cm compared to closer spacing of 30 cm × 10 cm during summer at Junagarh, Gujarat, India. The nodulation on roots of mungbean plant was found to increase from 30 DAS to 45 DAS, and that was declined thereafter to 60 DAS due to withering and drying of nodules. The number of nodules/plant at 45 DAS was gradually increased with delay in sowing from 15 February (17.1/plant) to 4 April (23.1/plant). Mid-March sown mungbean (11 March) recorded highest dry matter yield at 60 DAS (301.0 g/m^2) being at par with early April sown crop. Close row spacing (25 cm) resulted in greater DM production at 60 DAS (284.9 g/m^2 *vs.* 267.7 g/m^2) compared to wider row spacing (30 cm). 'Pant Mung 5' accumulated greater aerial DM at 60 DAS (285.1 g/m^2 *vs.* 267.5 g/m^2) than Samrat in the study. The CGR was increased gradually with delay in sowing from 15 February (6.62 g/m^2/day) to 4 April (8.78 g/m^2/day), but no significant effect of row spacing was noted on CGR.

Grain yield, stover yield and harvest index

Mungbean sown on 11 March produced the highest grain yield (684.3 kg/ha), which was 14.5% and 68.8% greater grain yield over 15 February, 4 April sowings, respectively (Table 2). The finding indicated that first fortnight of March might be optimum for

Table 2. Effect of sowing date, row spacing and variety on yield attributes and yield of summer mungbean

Treatment	Plant height (cm)	No. of branches/ plant	No. of nodules/ plant at 45 DAS	Dry matter accumulation (g/m^2) at 60 DAS	Crop growth rate (g/m^2/ day)		Grain yield (kg/ha)	Stover yield (kg/ha)	Harvest index
					30-45 DAS	45-60 DAS			
Sowing date									
15 February	44.6	5.44	17.1	227.6	5.47	6.62	597.8	1798.6	25.3
11 March	54.7	7.58	21.3	301.0	6.59	8.42	684.3	2486.7	22.9
4 April	55.6	8.02	23.1	300.2	4.94	8.78	405.5	2572.4	13.7
CD (P=0.05)	0.74	1.57	2.25	8.83	1.67	1.51	27.98	714.3	4.75
Row spacing									
30 cm	49.4	7.05	20.9	267.7	5.60	7.77	542.1	2156.1	21.0
25 cm	53.8	6.97	20.1	284.9	5.74	8.11	583.0	2415.7	20.2
CD (P=0.05)	0.29	NS	NS	12.00	0.74	1.02	32.13	217.2	NS
Variety									
'Samrat'	50.4	6.98	19.1	267.5	5.38	7.57	532.7	2178.2	20.1
'Pant Mung'	52.8	7.04	21.8	285.1	5.96	8.31	592.4	2393.6	21.2
CD (P=0.05)	0.50	NS	3.25	14.55	0.94	1.38	32.51	NS	NS

sowing of mungbean for realization of higher yield, and further delay caused reduction in yield during summer season in New Alluvial Zone of West Bengal. Close row spacing (25 cm) resulted in greater grain (580.0 kg/ha) compared to wider one (30 cm); while 'Pant Mung 5' yielded greater (592.4 kg/ha *vs.* 532.7 kg/ha) over 'Samrat'.

With delay in sowing from 15 February to 4 April, the stover yield was increased progressively from 1798.6 to 2572.4 kg/ha in the experiment. The trend could be supported by the growth attributes like plant height (44.6 cm vs. 55.6 cm), branching habit (544/ plant vs. 802 /plant) and dry matter accumulation (227.6 g/m^2 vs. 300.2 g/m^2) of the crop. Miah *et al.* (2019) reported similar findings, where late sowing (11 April) of five mungbean varieties resulted in maximum stover yield compared to earlier sowings (20 February, 2 March, 22 March and 1 April) at Mymensingh, Bangladesh. The greater stover yield was obtained from closely spaced crop (2415.7 kg/ ha) mainly due to greater plant population compared to widely spaced one (2156.1 kg/ha). Mid-February sown mungbean recorded maximum harvest index (25.3%), which was gradually decreased to 11 March (22.9%) and 4 April (13.7%) sowing in the study.

The phenological development of mungbean could be summerized by the days required from sowing to emergence, flower initiation, pod initiation, end of flowering, end of pod formation and maturity as 6.1, 33.5, 41.7, 50.8, 59.9 and 72.8, respectively. Sowings in mid-March and early April resulted in better vegetative growth in terms of plant height, branching habit and dry matter production due to high temperature compared to early sowing in mid-Fabruary.The duration of mungbean was shortened by 6.7 days with delay in sowing from 15 February (76.0 days) to 4 April (69.3 days), but mid-March sowing recorded the highest grain yield (684.3 kg/ha). Close row spacing (25 cm) resulted in greater grain (580.0 kg/ha) and straw yield (2415.7 kg/ha); while 'Pant Mung 5' accumulated greater total GDD (1214°C day), HTU (9443°C day hour) and PTU (15308°C day hour) for entire life cycle, and yielded higher (684.3 kg/ha) over 'Samrat'.

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