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Differences in seed vigour traits between desi (pigmented) and kabuli (non-pigmented) ecotypes of chickpea (*Cicer arietinum*) and its association with field emergence

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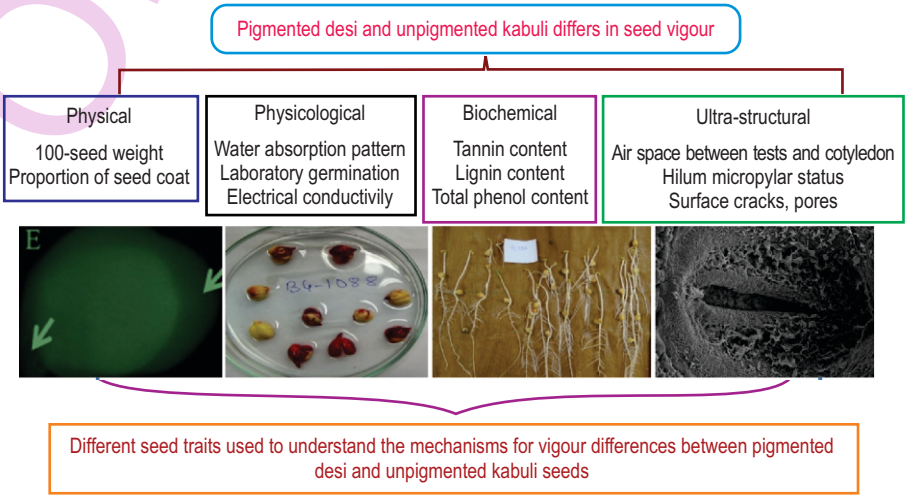
Abstract

Aim: Pigmented (desi) and non pigmented (kabuli) cultivars of chickpea are known to differ in seed vigour. Therefore, the main objective of the study was to understand the mechanisms for such vigour differences and to identify the important seed coat and seed related vigour traits that makes the coloured desi seeds more vigorous then unpigmented kabuli seeds.

Methodology: Twenty two chickpea genotypes differing in seed coat colour were included in the experiment. Field emergence and electrical conductivity of seed leachate was used as vigour indicator. Hundred-seed weight, proportion of seed coat, laboratory germination, electrical conductivity, water imbibition pattern, tannin, lignin and total phenol content, presence or absence of air space between seed coat and cotyledon and status of hilum-micropylar region were studied to understand the mechanism for vigour differences between pigmented desi and unpigmented kabuli genotypes.

Results: Despite a high laboratory germination (>89%) of all cultivars, unpigmented kabuli genotypes recorded low (39-69%) FE then pigmented desi genotypes (64-87%). Rapid rate of water imbibition (111.86-145.09%), lower proportion of seed coat (4.76-6.78%), greater electrical conductivity of seed leachate (49-172 $\mu S\ cm^{-1}\ g^{-1}$), low content of lignin (0.74-2.41), tannin (0.18-1.09 $\mu g\ mg^{-1}$) and total phenol (1.66-5.58 $\mu g\ mg^{-1}$) was associated with low field emergence in unpigmented kabuli types. Besides, air space between seed coat and cotyledon, open hilum-micropylar region, less polyphenolic content and low proportion of seed coat potentially describe the rapid water uptake by unpigmented kabuli genotypes making them vulnerable to imbibitional damage.

Interpretation: Rather than laboratory germination, electrical conductivity may be used as an indicator for determining field emergence in chickpea. Screening/ developing unpigmented kabuli genotypes with seeds having lower rate of water imbibition could be a promising way to enable seed vigour improvement in chickpea.



Introduction

Chickpea (*Cicer arietinum*) is an important food legume, currently grown in over 50 countries and consumed in over 120 countries. India is the leading chickpea producing country with 68% share in global chickpea production (FAO, 2014). Two types of chickpea *i.e.* desi and kabuli are well recognized. Desi types are small seeded with pigmented seed coat, while kabuli types are non-pigmented/beige colour and are large seeded. Seed coat colour is imparted by the presence of phenolic compounds (tannin) in the seed coat (Caldes and Blair, 2009), which are also known to have antimicrobial, antifungal properties and also protects the seed from precocious germination and insect pests. Rapid establishment and uniform plant stand under agro-ecological regions is required for realizing high yields, which in turn depends on seed vigour. Seeds that can germinate quickly to produce normal and vigorous seedlings show little sensitivity to external factors, which enables them to establish in a wide range of agro-climatic conditions (Corbineau and Come, 2006; Mohammadi *et al*., 2011). Vigour differences in legume crops were reported to be associated with seed coat pigmentation, examples were reported in french beans (Dickson and Petzoldt, 1988), fababean (Kantar *et al*., 1996), flax (Saeidi, 2008) and cowpea (Peksen *et al.*, 2004). Poor seed vigour in these crops is often linked with transverse cracking of the cotyledon and seed coat, open hilum, imbibitional damage resulting from rapid uptake of water and increased solute leakage from seeds. In kabuli types, Yadav and Sharma (2001) concluded that poor plant establishment was due to delicate seed coat. Detailed studies that examine the important seed characteristics contributing to seed vigour are lacking, particularly in chickpea. Given the significance of seed vigour in field emergence, in the present study key seed traits that contribute to difference in seed vigour between pigmented desi and non-pigmented kabuli types were analyzed. These seed traits include physical, physiological, physico -chemical and ultra-structural properties of the two market types.

Materials and Methods

Twenty two genotypes of chickpea, 11 each in desi (G 118, G 7, G 50, G 229, G 39, G 95, G 53, G 66, G 18, G 56 and G 148) and kabuli (G 71, G 227, G 149, G 232, G 235, G 137, G 233, G 144, CSJK 21, BG 1105 and BG 1088) differing in testa colour and seed size were used in this study.

Three replications of 100 seeds each were taken for 100-seed weight determination. Seed coat from seeds were removed carefully after soaking in distill water for 4-5 hrs. Seed coat and cotyledons were then dried at 80ºC for 24 hrs and weighed separately. The proportion of seed coat was than calculated. Four to six whole seeds from each genotype were taken randomly for X-ray photography (Faxitron X-ray) after soaking it in distilled water for 30 min to examine the air space between seed coat and cotyledons. Scanning Electron Microscopy (SEM) was carried out on single seeds of kabuli BG-1088 and desi G-229 using S-3400N (Hitachi) to examine for variation in seed morphology. BG-1088 imbibed maximum water for the initial one hour of imbibition and G-229 imbibed the least, therefore these two genotypes were studied under scanning electron microscope to understand the differences in seed structure.

Laboratory germination (%), vigour indices and field emergence (%) : Three replicates, each of 100 seeds, were kept for germination in an incubator at 25 ºC in rolled germination towels. After 7 days of incubation, normal seedlings were counted (ISTA, 2011) and percentage germination calculated. The root and shoot lengths of ten seedlings were measured and dried in an oven at 80 ºC for 24 hrs to determine the seedling dry weight. Subsequently, vigour indices I and II were calculated following the protocol of (Abdul Baki and Anderson (1973).

Field emergence was tested by hand-sowing of seeds in 10 m rows in three replications with a spacing of 30 X 15 cm between row and plant respectively. The number of seedlings emerged after 20 days were counted and expressed as field emergence percentage.

Imbibition pattern and electrical conductivity : Fifty seeds of each sample were weighed and soaked in 250 ml de-ionized water at 20°C. Seeds were re-weighed after1, 4, 8, 12, 16 and 24 hrs. The percentage increase in water uptake was calculated. After 24 hrs, electrical conductivity of steep water was measured by conductivity meter and expressed in μS cm^{-1} g^{-1} of seed. The test was conducted in three replicates.

Determination of lignin content: Ground samples were extracted in NaOH for 12-16 hrs at 70-80 ºC. To 0.8ml of extracts 0.8ml each of 0.1 M sodium phosphate buffer and 0.1N NaOH was added and pH of 7.0 and 12.3 was maintained respectively and absorbance was measured in UV-VIS spectrophotometer (Simadzu) at 245 and 350 nm. The amount of lignin was calculated by the difference between A 245 (pH 7.0) and *A 350* (pH 12.3) (Thimmaiah, 1999).

Determination of tannin content: Ground material was extracted in 70% acetone in electric shaker for 2hr followed by filtering it through Whatmaan filter paper No. 1. Folin-Ciocalteu method was used for determination of tannin content which includes spectrophotometric reading at 725 nm (Shimadzu). 1 ml of sample, 75 ml distill water, 5 ml of Folin-Ciocalteu reagent and 10 ml of sodium carbonate solution was mixed and absorbance was measured after 30 minutes (Thimmaiah, 1999).

Determination of total phenolic content : 0.1 g powder was extracted in a 2 ml microfuge tube with 1 ml methanol/water (70:30, v/v) (Xu and Chang, 2007). The mixture was shaken at 300 rpm at ambient temperature for 3 hr and extracted for an additional 16 hr in the dark overnight. The extracts were

centrifuged at 12000 rpm for 15 min, and the supernatants were removed into new tubes. Extracts were kept in dark at 4°C until determination of total phenol content. Total phenol content was determined by the Folin–Ciocalteu assay (Singleton and Rossi, 1965).

Statistical analysis : Data were analyzed statistically using SPSS 16.0 software package. Duncans multiple range test (DMRT) was performed at p= 0.05 to test the significance of differences.

Results and Discussion

Laboratory germination of seeds exceeded 89% (Fig. 1A) with average vigour indices (VI)-I and II of 2411.09 and 2100.64; 30.04 and 43.54 for pigmented desi and non-pigmented kabuli genotypes, respectively (Fig. 1B). In spite of high laboratory germination and vigour indices, emergence in the field was low for seeds of non-pigmented kabuli type (≤69%). Kabuli genotype CSJK-21 recorded field emergence of only 39%. All the pigmented desi genotypes, except for G-229 (64%) had high

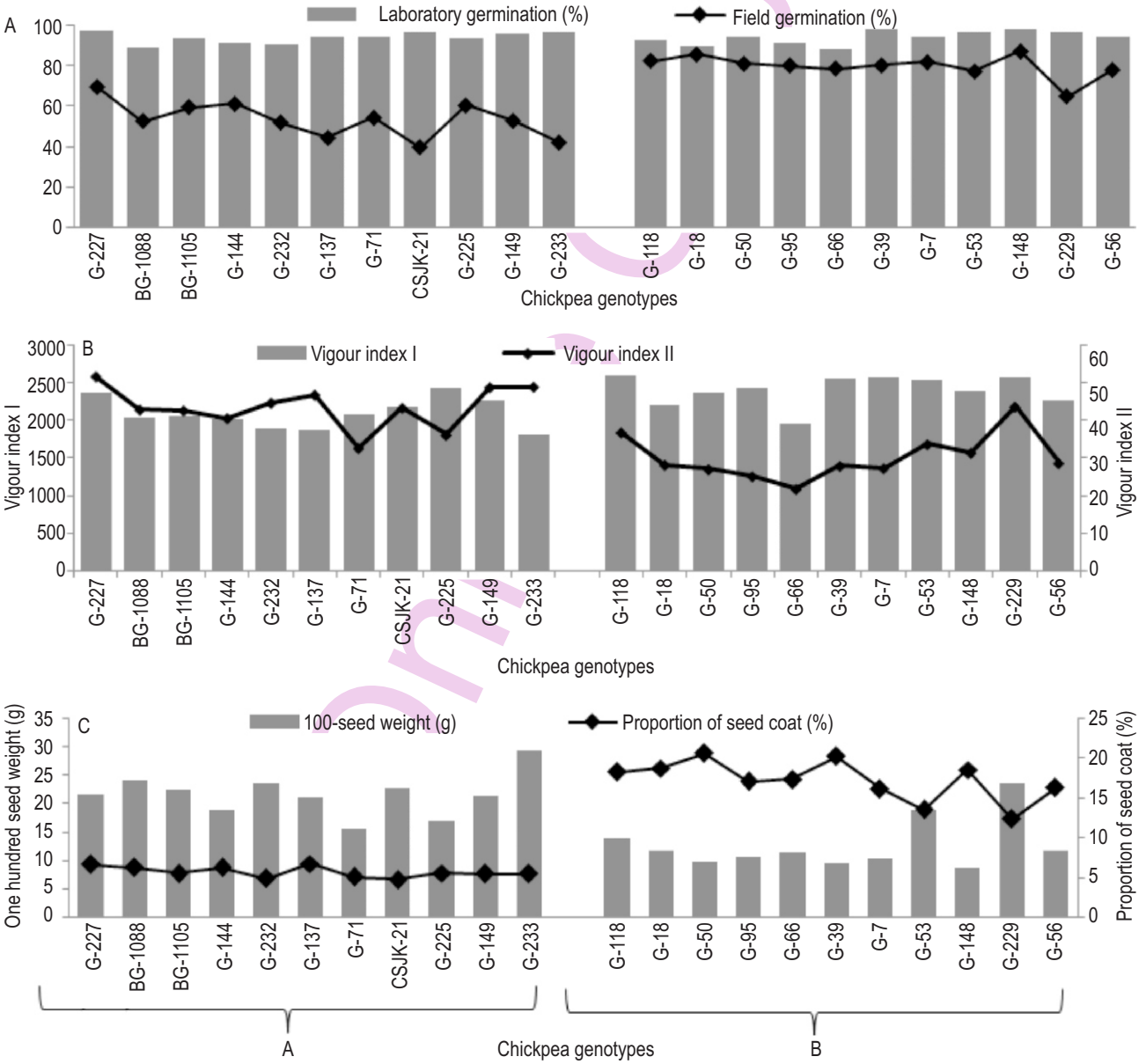


Fig. 1 : (A) Laboratory germination and field emergence, (B) Vigour index I and II and (C) one hundred seed weight and proportion of seed coat of twenty two chickpea genotypes. Genotypes represented by 'A' are of non-pigmented kabuli type and genotypes represented by 'B' are pigmented desi genotypes

emergence percentage (≥80%). Although all the genotypes showed high laboratory germination and vigour indices, there were significant differences in the field emergence of the seedlings. In general, pigmented desi types showed better emergence than non-pigmented kabuli types indicating that they differed in seed vigour. Similar findings that relate poor field emergence with high laboratory germination has been reported in white seeded fababean (Kantar *et al*., 1996) and white seeded cowpea (Peksen *et al*., 2004).

In legume seed, outer cover or testa acts as a modulator between the internal seed structure and the environment and is an important factor that determines field emergence and vigour (Souza and Filho, 2001), either by maintaining the integrity of internal seed components, by protecting the embryo, allowing the exchange of gases between embryo and the environment and by regulating the imbibition process. Therefore, in the present study it was examined whether the vigour differences in pigmented desi and non-pigmented kabuli chickpea in terms of field emergence

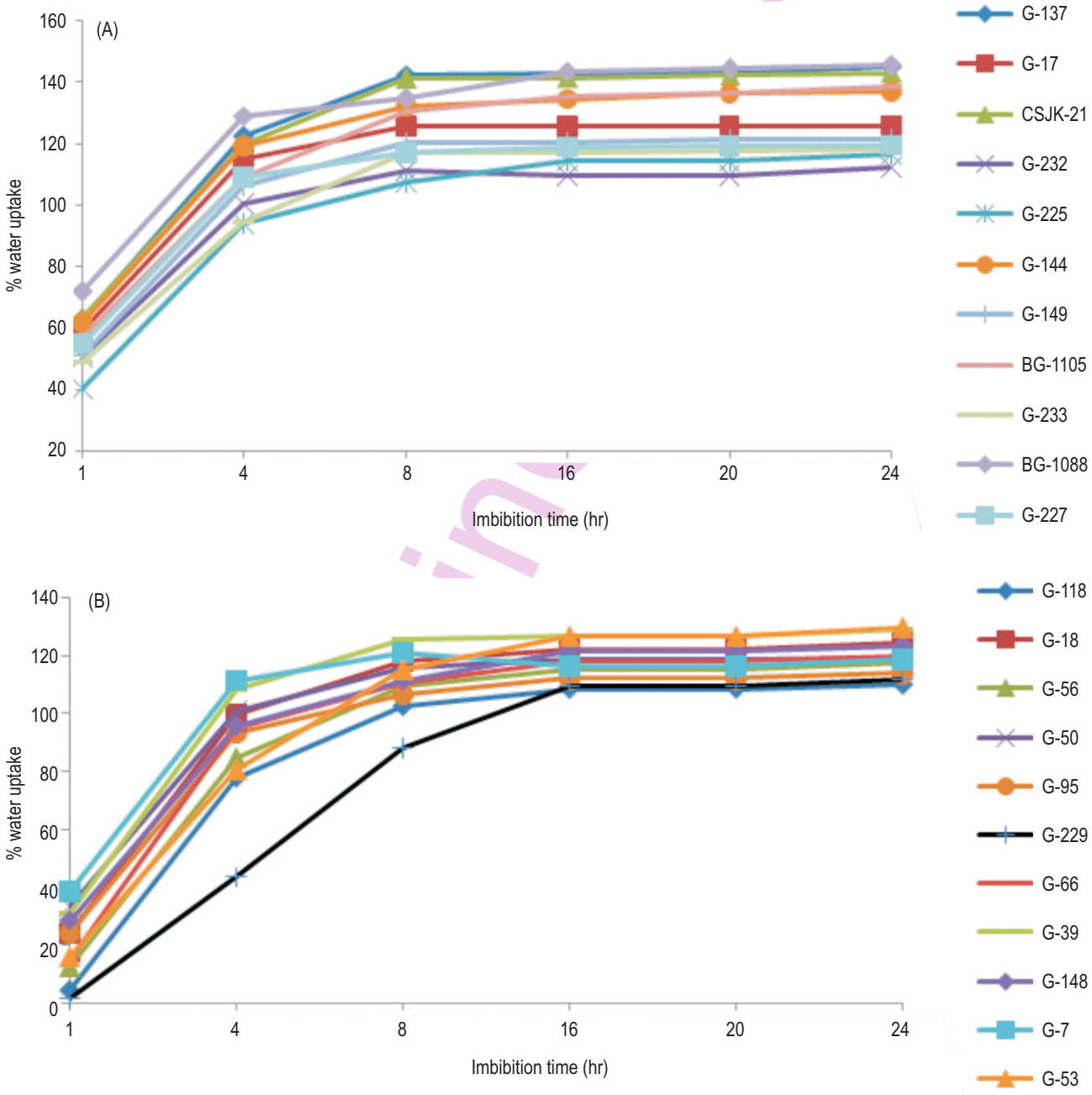


Fig. 2 : (A) Cumulative water absorption time course during imbibition of non-pigmented kabuli and (B) pigmented desi (B) genotypes

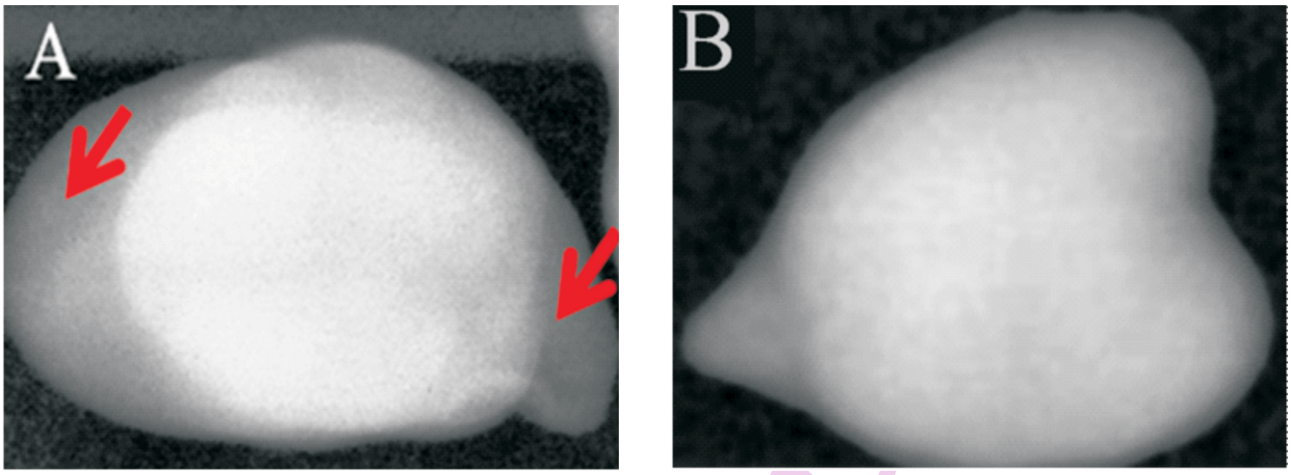


Fig. 3 : (A) Existence of air space between seed coat and cotyledon as revealed by X-Ray photography in non-pigmented kabuli genotype BG-1088 and (B) absence of such space in pigmented desi genotype G-66

Table 1 : EC values of steep water as recorded after 24 hrs of imbibition

Kabuli genotypes (non-pigmented)	EC (µS cm^{-1} g^{-1})	Desi genotypes (pigmented)	EC (µS cm^{-1} g^{-1})
G-227	65.67^{abcde}	G-118	36.33^{a}
BG-1088	140.00^{gh}	G-18	43.00^{ab}
BG-1105	123.33^{fgh}	G-50	37.33^{ab}
G-144	101.33^{cdefg}	G-95	27.33^{a}
G-232	107.33^{defg}	G-66	43.00^{ab}
G-137	172.00^{h}	G-39	36.00^{a}
G-71	$76.33^{abcdefg}$	G-7	64.33^{abcd}
CSJK-21	147.00^{gh}	G-53	80.33^{abcdef}
G-225	49.00^{abc}	G-148	43.00^{ab}
G-149	116.33^{defg}	G-229	64.00^{abcd}
G-233	120.00^{efgh}	G-56	46.33^{abc}
Average	110.76		47.36

Mean followed by similar alphabets indicates non-significant differences between the values at 0.05 level of significance

was due to differences in the physical testa structure, physiological, chemical constituents or ultra structural properties.

Significant differences existed for hundred-seed weight and proportion of seed coat among the chickpea genotypes studied. The hundred-seed weight of non-pigmented kabuli types ranged from 15.89g to 30.82g, while pigmented desi type showed a variation ranging between 8.16g and 22.17g. Coloured desi genotypes had almost three times higher seed coat ratio than white coloured kabuli types. Pigmented desi genotype G-50 had maximum proportion of seed coat (20.58%), while minimum (4.76%) was observed for non-pigmented kabuli type CSJK-21 (Fig. 1C). Content of seed coat is an indirect measure of seed coat thickness which is described as a physical defensive trait (Zhang *et al.,* 2016). Further, a significant negative (-0.84**) association observed between hundred-seed weight and proportion of seed coat implying that non-pigmented kabuli type being bold seeded tends to have less physical defensive trait or seed coat. Seed coat regulates the water uptake process by seeds during imbibition (Chachalis and Smith, 2001). Significant differences were observed between non-pigmented kabuli and pigmented desi genotypes in terms of water uptake. Percent increase in water uptake of coloured desi genotypes ranged from 109.81% (G-118) to 128.98% (G-39), while in case of un-pigmented kabuli types it varied between 111.86% (G-232) and 145.09% (BG-1088) at 24th hr. Moreover, desi and kabuli seeds differed in water absorption rate depending on the imbibition time. For the initial first hour of imbibition, the water uptake of most of the coloured desi genotypes was less than 40% with some genotypes like G-118 and G-229 imbibing less than 5% water. On the contrary, entire non-pigmented kabuli types imbibed more than 40% water, and notably, the genotypes G-137, G-144, CSJK-21 and BG-1088 had more than 60% water uptake. It is important to note that the non-pigmented kabuli genotypes reached their maximum water

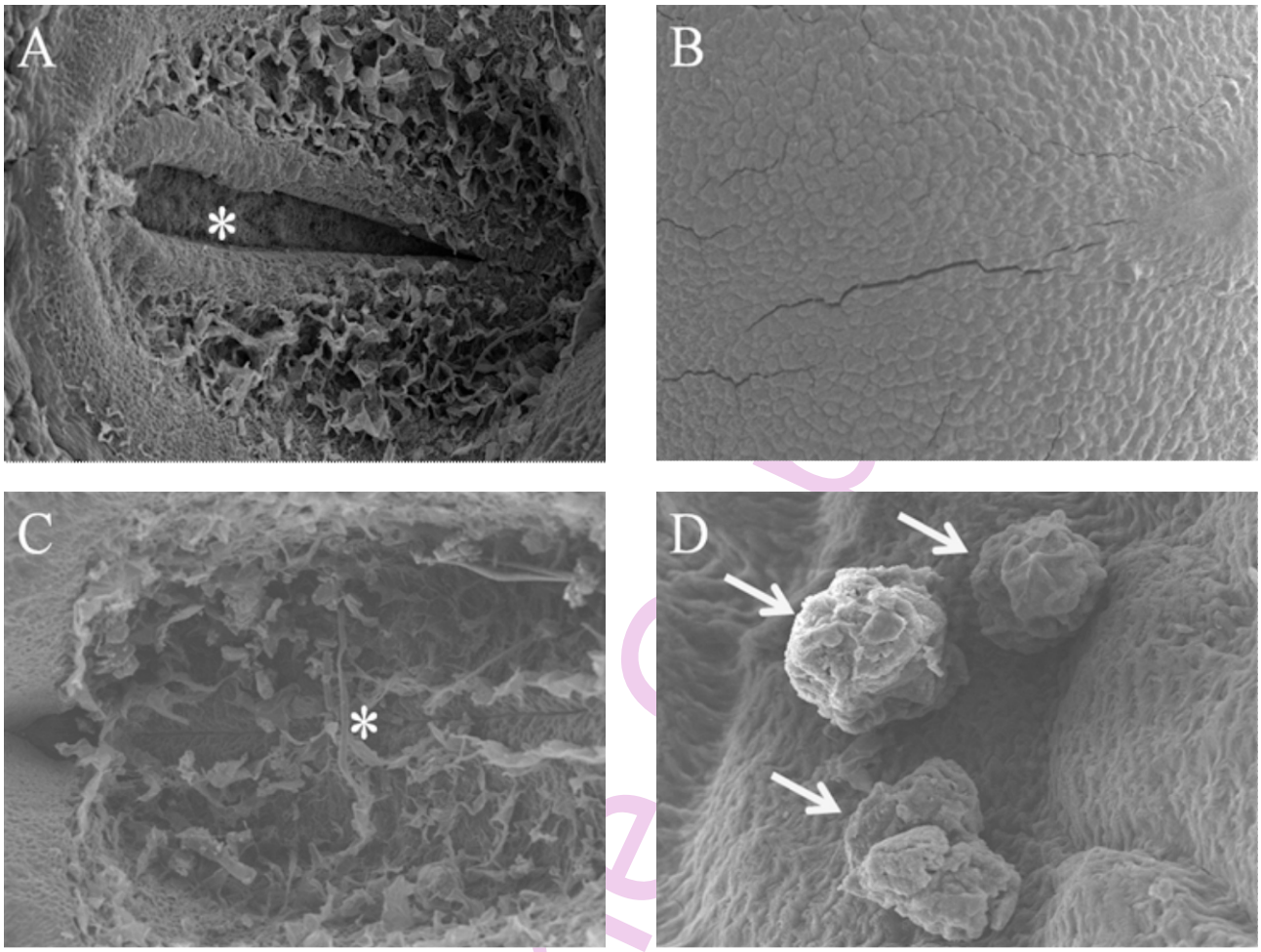


Fig. 4 : Scanning electron micrographs of chickpea seeds; (A) 'Kabuli (non-pigmented)-BG-1088'. The hilum-micropyle region is open (asterisk). (B) Surface cracks in the seeds of 'Kabuli-BG-1088'. (C) Closed hilum-micropyle region in 'Desi (pigmented)-G-229'. (D) Presence of surface deposits (arrows) in 'Desi-G-229'. Scale bars: A = 500 µm, B = 1mm C = 300 µm and D = 100 µm

imbibing capacity between 4-8 hrs, whereas pigmented desi genotypes continued to imbibe water even up to 16 h of imbibition (Fig. 2). Further, electrical conductivity values of the steep water were also found to vary significantly between as well as within the pigmented desi and non-pigmented kabuli genotypes (Table 1). Majority of the non-pigmented kabuli genotypes had leachate conductivities greater than 100 µS cm^{-1} g^{-1} seed, while all other coloured desi genotype had low leachate conductivity (≤64.33µ S cm^{-1} g^{-1} seed) except for G-53 (80.33 µS cm^{-1} g^{-1} seed).

Negative and significant correlation (r = -0.438*) was found between field emergence and water absorption rate. Similar negative correlation between WAR and field emergence was also observed in other legume crop such as cowpea (Peksen *et al.,* 2004); common bean (Borji 2007) and fababean (Peksen 2007). Permeability of testa, proportion of seed coat, presence of cracks and pores in the seed coat, hilum status, degree of adherence of testa to cotyledons, chemical composition of seed coat are some of the characters that regulate entry of water into the seed (Peksen *et al*., 2004). Slow water uptake by desi types is attributed to the coloured seed coat which remains tightly adhered to the cotyledons in contrast to the non-pigmented kabuli seeds that carry large air space between testa and cotyledon as revealed by X-ray radiography (Fig. 3). Therefore, in non-pigmented kabuli types, the loose adherence of testa enabled free movement of water throughout the seed. In contrast, a tight adherence of testa with cotyledon in coloured desi types restricted the free flow of water between the cotyledons and testa, finally leading to slower imbibition and lesser amount of electrolyte leaching out. Similar results were reported earlier in legume seeds having white testa (Fengshen *et al*., 2004).

Non-pigmented kabuli genotype BG-1088 recorded the highest water uptake while pigmented desi G-229 recorded the lowest in the first hour of imbibition. Therefore, these genotypes were examined under scanning electron microscope for

Table 2 : Variation in lignin, tannin and TPC among chickpea genotypes

Kabuli genotype (non-pigmented)	Lignin content (OD245nm-OD350nm)	Tannin (µg mg^{-1})	TPC (µg mg^{-1})	Desi genotype (pigmented)	Lignin content (OD_{245nm}-OD_{350nm})	Tannin (µg mg^{-1})	TPC (µg mg^{-1})
G-227	0.95^{d}	0.50^{a}	1.68^{a}	G-118	2.73^{l}	6.17^{d}	7.22^{d}
BG-1088	0.89^{cd}	0.27^{a}	2.73^{a}	G-18	2.49^{j}	4.93^{cd}	9.91^{e}
BG-1105	0.92^{cd}	0.54^{a}	1.66^{a}	G-50	2.54^{j}	10.53^{f}	16.84^{h}
G-144	0.90^{cd}	0.26^{a}	2.08^{a}	G-95	2.90^{no}	3.57^{b}	20.07^{i}
G-232	0.92^{cd}	0.07^{a}	2.58^{a}	G-66	2.63^{k}	5.78^{d}	33.53^{k}
G-137	0.74^{b}	0.78^{a}	4.48^{bc}	G-39	1.28^{e}	11.74^{f}	16.47^{h}
G-71	1.12^{e}	0.35^{a}	2.97^{ab}	G-7	2.84^{m}	5.56^{d}	8.44^{d}
CSJK-21	0.60^{a}	0.65^{a}	1.75^{a}	G-53	2.96^{o}	11.65^{f}	23.85^{j}
G-225	1.34^{f}	0.21^{a}	2.19^{a}	G-148	3.09^{p}	5.84^{d}	12.29^{f}
G-149	2.41^{i}	1.09^{a}	2.99^{ab}	G-229	2.88^{mn}	3.92^{bc}	8.11^{d}
G-233	0.87^{c}	0.18^{a}	5.58^{c}	G-56	2.31^{h}	8.08^{e}	47.77^{ab}
Average	1.06	0.44	2.79		2.60	7.07	18.60

Mean followed by the similar alphabets indicates non-significant difference between the values at 0.05 level of significance

differences in seed coat structure if any. BG-1088 and G-229 revealed their overall similarity concerning the structure of their seed coats, each having a micropyle, a hilum, a raphe and an extra-hilar region. In dry seeds, the micropylar-hilum region was widely open as observed with scanning electron microscope in BG-1088 (Fig. 4A) which otherwise was found closed in G-229 (Fig. 4C). Presence of surface cracks was consistent in BG-1088 (Fig. 4B), but such cracks were absent in G-229 and instead G-229 showed some surface deposits (Fig. 4D). Presence of such deposits on the seed surface is in accordance with the findings reported in case of impermeable soybean seeds (Fengshen *et al*., 2004).

The chemical content of seed coat also determines the water uptake behavior of seeds. Significant genotypic variation was observed in lignin, tannin and total phenol content. As expected, the lignin, tannin and the total phenol content were more in the seeds of desi types. The average lignin, tannin and total phenol content of pigmented desi and non-pigmented kabuli seeds were 2.60 and 1.06; 7.07µg mg^{-1} and 0.44 µg mg^{-1}; 18.60 µg mg^{-1} and 2.79 µg mg^{-1}, respectively. Genotypes G-148, G-39 and G-56 recorded highest lignin (3.09), tannin (33.53 µg mg^{-1}) and total phenol content (47.77 µg mg^{-1}), respectively (Table 2). This finding remains in good agreement with an earlier report that showed higher content of phenolics in coloured chickpea seeds than beige seeds (Segav *et al.,* 2010). A very high and significant correlation was observed between field emergence with lignin (0.77**), tannin (0.75**) and total phenol content (0.62**). This observation finds concordance with Saeidi and Rowland (2000) who reported higher phenolic and tannin content in the seed coat of brown seeded flax as compared to yellow seeds. In a similar manner, enhanced vigour and field emergence in brown-seeded flax was reported to be associated with higher phenolic (tannin) content in its seed coat (Saeidi and Rowland, 2000). Based on the results obtained in the study, the discrepancies in water absorption rate and field emergence between non-pigmented kabuli and pigmented desi genotypes might be accounted to the presence of high amount of such compounds in the seed coat of later. Such compounds may affect field emergence either by reducing the rapid uptake of water by seeds thus, preventing them from soaking injury and transverse cracking of the cotyledons which otherwise leads to high solute leakage and reduces respiration and translocation rates of reserves from cotyledons to the growing axis of the seeds (Freeman, 1995). Ultimately, it damages the embryo thereby affects in the field emergence or the seed metabolites leached out of seeds due to soaking injury or imbibitional damage serve as an entry point for fungal invasion and cause pre-emergence decaying of seed, thus exerting impact on the field emergence. Such compounds are known to have antibiotic, anti microbial and anti fungal properties (Farahin *et al*., 2016). Thus, such compounds protect the seeds from soil fungus and enable a uniform emergence. Wu *et al*. (2010) reported that tannin reduced the conidial growth of *Fusarium oxysporium* by 52.3%.

Water absorption rate showed a wide variation within and between pigmented desi and non-pigmented kabuli ecotypes. Therefore, good opportunities exist for improving the seed quality and vigour of white kabuli cultivars through selection following appropriate breeding programmes. Precise identification of seed coat trait(s) associated with pigmentation that confer slow imbibition would allow its introduction and subsequent selection among breeding lines having non-pigmented testa. In parallel, emphasis should also be given to screen kabuli types with comparatively higher contents of lignin, tannin and total phenolic compounds along with slow rate of imbibition. The experimental results reported here will certainly guide chickpea breeders for chickpea improvement.

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