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#### Abstract

Sugarcane (Saccharum officinarum L.) is grown in both tropical as well as sub tropical regions and the diseases are one among the major constraints in crop production. The severity of the diseases are mainly influenced by the varieties, crop stage and major weather parameters such as temperature and precipitation. Apart from stalk pathogens such as red rot, wilt and smut, the foliar pathogens of sugarcane especially rust causes substantial vield losses due to disease epidemics in susceptible varieties. The disease has been reported in most of the sugarcane growing countries especially in tropical regions. The uredospores of rust are carried away in wind current and are capable of causing fresh infection in sugarcane plants across the fields. In India, the rust severity is restricted to peninsular regions in Tamil Nadu, Karnataka, Andhra Pradesh, Telangana, Gujarat and Maharashtra and sub tropical states are relatively free from rust diseases due to unfavourable climate for rust pathogens. Though the disease has been reported more than 100 years ago in India, information on sugarcane rust is scanty hence an attempt has been made to review sugarcane rusts and their epiphytotics in the past and their impact on country's economy and importance of rust resistant varieties in containing the disease. Major emphasis was given on the pathogen biology, variability and development of new races in sugarcane growing regions, influence of weather factors in host pathogen interaction and breakdown of resistant varieties, trans-oceanic movement of uredospores, sources of rust resistance among world sugarcane germplasm and Bru genes and their contribution in brown rust resistance and management of sugarcane rusts using chemicals and host resistance.

Keywords : Sugarcane, brown rust, orange rust, Puccinia melanocephala, Puccinia kuehnii

#### Introduction

Sugarcane, an important cash crop and the source for 80% of the crystal sugar in the world is grown over more than 110 countries of both tropical as well as subtropical regions upto 30 °N and 35 °C latitudes on both sides of equator. In the world, India ranks second both in area and production after Brazil (FAO 2019). Diseases are one of the major constraints in obtaining higher productivity in sugarcane and more than 55 diseases have been reported from India (Rao et al. 2002). Among the foliar disease of sugarcane, rust is an important fungal disease distributed worldwide in more than 60 countries and has caused economic losses to crop cultivation in several countries (EPPO 2019). Based on symptoms of infection and colour of uredospores, three types of rusts are known in sugarcane till date. There were two species of Puccinia reported to attack sugarcane throughout the world and the interaction results in rusty appearance on leaves. The brown rust is caused by Puccinia melanocephala (Syd. & P.Syd) and orange rust incited by P. kuehnii (W.Kruger) E.J. Butler (Braithwaite et al. 2009). During 2008, a new rust of sugarcane known as African sugarcane rust was first reported to infect sugarcane in Swaziland of South Africa. The rust renamed as tawny rust caused by Macruropyxis fulva sp. nov. increasing the number of rust species to three infecting sugarcane (Martin et al. 2017). Of these three rusts, brown rust is reported from many countries followed by orange rusts in causing economic losses. However,

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the tawny rust is restricted only to South African continent till date (Rutherford 2018).

# Historical perspectives of sugarcane rusts and their impact

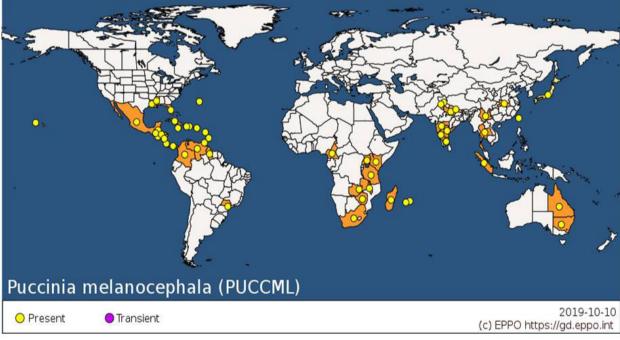
The rusts are obligate pathogens, survive only on living tissues. Their short life cycle and the wind borne nature of uredospores play a major role in spreading the rusts across continents. The continuous presence of sugarcane in the field as main crop or ratoon, growing single susceptible variety over a larger areas and conducive environmental factors for the pathogens favour the build of inoculum and development of rust epidemics. Both brown and orange rusts have caused severe epidemics and that caused yield decline in popular varieties or their removal from cultivation in many countries. The yield loss varied from 10 to 20% and under severe conditions the yield loss reached up to 50% in many countries (Bernard 1980a).

#### **Brown rust**

Brown rust has a wide distribution and reported

in many sugarcane growing countries (Fig. 1). During 1980's, more than 29 sugarcane growing countries in the world have reported the presence of brown rust and the number has increased to more than 40 during 2019 (Egan 1980; EPPO 2019a). Many inaccuracies were also observed in the reports on occurrence of sugarcane rust in different countries due to misidentification of the pathogen. There were attempts earlier to review worldwide distribution of sugarcane rust from different countries (Egan 1980; Rutherford 2018; Selvakumar and Viswanathan 2018).

The brown rust was first reported in 1907 in India on *Erianthus ravennae* as *P. melanocephala*. The *P. erianthi* on *E. rufipilus* and *P. sacchari* on sugarcane are synonyms to this pathogen (Cummins 1971). In India, rusts have been reported from Andhra Pradesh, Kerala, Maharashtra, Punjab, Tamil Nadu and Uttar Pradesh (Jamaluddin et al. 2004; Prasher et al. 2015). In India, during 1949 crop season, a severe outbreak of brown rust of sugarcane was observed during September



**Fig. 1.** Distribution map of *P. melanocephala* (With permission from EPPO. Source: https://gd.eppo.int/taxon/ PUCCML/distribution)

- October on a high yielding popular variety Co 475, affecting more than 50% of areas in Belapur, Kopergaon, Belwandi, Ahmednagar, Bramati, Akluj and other Deccan canal tracts, hence it was withdrawn from the cultivation in Maharashtra state (Patel et al.1950; Chona and Munjal 1950). During 1955-56, a rust epidemic was observed on a variety CoS 510 in sugarcane growing regions of Himalayan foot hills in Uttar Pradesh (Vasudeva 1956). Later, the cvs CoS 510 and Co 876 were replaced due to their brown rust susceptibility in India (Tiwari and Singh 1962). The authors have witnessed severe rust epidemics on the cv Co 0323 in Chamrajnagar and Mysore districts in Karnataka during 2016-17 season, reducing the crop yield significantly. The variety Co 0323 planted during February showed less rust severity whereas, June-July planted crop became vulnerable for the disease due to its young growth phase and the favourable environment for expression of rust severity in the area (Selvakumar and Viswanathan 2017). In the same region, the newly released variety CoVc 03165 have succumbed to severe brown rust especially in Koppa in Mandya district. Entire fields exhibited a rusty foliage and the variety could not be popularized in the region. Apart from this, other varieties under multiplication like Co 94008, Co 98005, CoC 671, Co 94012 and VSI 434 exhibited moderate to severe rust severity (Viswanathan, unpublished).

The brown rust was reported in Mozambique and Malagasy Republic by the early 1950's and later it spread to Kenya, Malawi, Tanzania, Uganda, Zimbabwe and Zambia in 1960's (Bock 1970; Logan, 1974; Martin, 1956; Siddiqi, 1969; Watson, 1965). The appearance of brown rust in eastern hemisphere was noticed in Japan, Taiwan, and Australia during 1975, 1977 and 1978 respectively (Hsieh et al. 1977; Ohtsu 1975; Purdy et al. 1983). In 1978, the brown rust was first observed in the Dominican Republic. In South America, the rust was reported in Colombia, Cauca Valley, Brazil during 1979, 1981 and 1986, respectively and it rapidly spread to Pernambuco and Alagoas states in Brazil within a short period (Victoria et al. 1984). In USA, the brown rust was reported in Florida, Louisiana and Hawaii during 1979, 1980 and 1982, respectively (Comstock et al.1982; Dean et al. 1979; Koike 1980).

During 1941, brown rust outbreak was noticed on the variety Co 301 in South Africa (Bailey 1979). During 1960's, the variety B 4362 grown on large areas in Angola and Cameroon in Africa suffered severe yield losses (Bernard 1978). In Cuba, the brown rust was first detected in September 1978 on the popular variety B 4362 which occupied 40% of cane growing areas of the country (Sandoval 1979). To curtail the spread of brown rust, rust resistant variety Ja60-5 was introduced in 1979 and replaced the cv B 4362. The variety Ja60-5 spread in a faster way and occupied 60% of sugarcane area during 1998 but there was a brown rust epidemic due to breakdown of resistance in year 1998 in Cuba (La et al. 2018). As a result, a sugarcane varietal policy was supported by the Agriculture Ministry of Cuba not to grow a single variety in more than 20% of the production area of the province to minimize the losses due to rust outbreak (La et al. 2018).

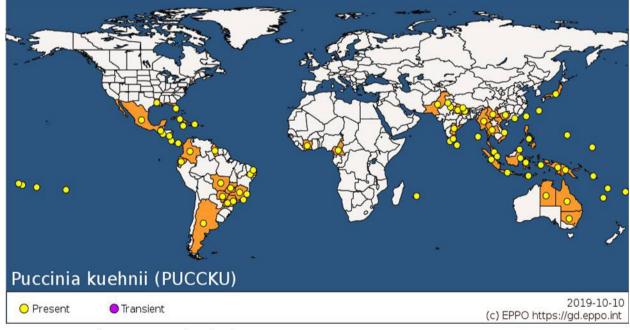
In Florida and Louisiana, 10 -50% yield loss have been reported due to brown rust in many popular varieties (Comstock et al. 1992; Raid and Comstock 2006). Many popular brown rust varieties such as CP 72-1210, CP 74-2005, and CP 78-1628 were withdrawn from cultivation after they become susceptible to brown rust in Florida due to breakdown of rust resistance by the appearance of new virulent strains (Raid 1989). During 1986, the variety CP 78-1247 was released and it behaved as moderately resistant in the field without showing any rust pustules on it and occupied a large area of 1292 ha within two years of its release in Florida. During 1988, a sudden outbreak of brown rust was noticed in entire south Florida state causing drying of more than 50% of top visible dewlap leaf in the variety CP 78-1247 (Raid 1988). During 1988, severe brown rust on the variety CP 78-1247 resulted in nearly 40 percent losses and of 20-25 percent on a very popular variety, CP 72-1210, occupying 60% of Florida's sugarcane acreage causing the economic loss of \$40 million (Raid and Comstock 2006). Two rust resistant varieties viz., CP 74-2005 and CL 73-239 were introduced in Florida in place of CP 72-1210 but they became susceptible in 1989 (Shine et al. 2005).

In Australia, brown rust was first noticed in 1978 on rust resistant varieties such as CP 44-101, Q 87, Q 110 and 67 N 1352 in North Queensland. The commercial varieties viz., Q 84, Q 90 and Q 105 derived from Co 775 recorded 10% yield loss (Ryan and Egan 1989). There was a reduction of 33% in cane tonnage and 31% in tonnes of sugar per hectare in Q 105 (Taylor et al. 1986).

#### **Orange rust**

The orange rust was first reported in 1890 by

Krüger in Java and Uromyces kuehnii and Uredo kuehnii are synonyms of this pathogen (Butler 1918; Kruger 1890; Wakker and Went 1898). Based on teliospores morphology, the pathogen was reclassified as Puccinia kuehnii (Butler 1918). Till the year 1980, more than 18 sugarcane growing countries have reported the occurrence of orange rust throughout the world (Egan 1980). Previously this rust was considered as a minor pathogen confined to India, China, Japan, Indonesia, the Philippines, New Guinea, Australia, Fiji and Samoa (Egan 1981). But the latest reports reveal that orange rust has been reported from more than 45 countries covering four continents Africa, America, Asia and Oceania (EPPO 2019b; Saumtally et al. 2011; Martin et al. 2017). In Africa, the orange rust has been reported in states of western and central Africa and the South African states are free from orange rust (Fig. 2). The orange rust was first observed in Florida on the variety CP 80-1743 during 2007 (Comstock et al. 2010). Subsequently, it was observed in other central American countries viz., Guatemala, Costa



**Fig. 2.** Distribution map of *P. kuehnii* (With permission from EPPO. Source : https://gd.eppo.int / taxon/ PUCCKU/distribution)

Rica, Nicaragua, El Salvador, Mexico, Brazil and Panama (Barbasso et al. 2010; Chavarría et al. 2009; Flores et al. 2009; Ovalle et al. 2008). After the detection of orange rust in Brazil in Sa<sup>o</sup>o Paulo State during 2009, it spread to new areas within 2 years of period causing 15 to 30 % loss in agricultural production in susceptible cultivars (Barbasso et al. 2010; Klosowski et al. 2013).

In Australia, the orange rust was considered as a minor disease till the year 2000. The higher rainfall coupled with warmer weather in Queensland, a sudden outbreak of an orange rust epidemic was noticed during 2000 due to the appearance of new virulent race. The popular variety Q124 which occupied 45% of cane area in Australia was severely affected due to breakdown of rust resistance. The sudden development and spread of orange rust during the epidemic resulted in a total losses of A\$150-210 million and yield loss of 38-40 % in the variety. An 'Orange Rust Task Force' (ORTF) was established in central Queensland to minimize the losses. The variety was quickly replaced with other resistant varieties such as Q 157, Q 170 and Q 190 (Magarey et al. 2001).

In Florida, , the orange rust was first observed on a very popular brown rust resistant variety CP 80-1743 covering one third of sugarcane crop area during 2007 (Comstock et al. 2010). The orange rust resistant varieties such as CP88-1762 and CP89-2143 were introduced but within a short period of time they also became susceptible to new rust variants in Florida. Due to the presence of both brown and orange rusts in Florida, the farmers preferred to grow high yielding brown rust susceptible varieties in the field with the help of fungicidal spraying. A high yielding variety CP96-1252, was preferred by the farmers though it was moderately susceptible to brown rust and occupied 29% of the sugarcane acreage in Florida in 2016 (VanWeelden et al. 2017; Raid et al. 2018; Rott 2018). During 2015-16 crop seasons, more than 90% of the sugarcane cultivars in the Florida farmer's fields were found to be susceptible to either of the rust pathogens (Raid et al. 2015).

In Brazil, the most popular variety RB 72454 with brown rust resistance was released in 1987 and more than 25 RB cultivars were derived from it. In 1995, the variety occupied 22.1% of the total area of sugarcane cultivation however the area reduced to 4.7% in 2010 due to mechanical harvesting and its susceptibility to orange rust (Daros et al. 2015; Gazaffi et al. 2016).

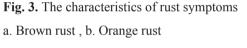
### Tawny rust

The newly described tawny rust was first discovered during 2008 on sugarcane variety N25 in Swaziland and later reported from Mozambique and Zimbabwe in South Africa (Martin et al.2013, 2015). In recent years, many varieties such as N12, N25, N31, N41, N43, N46, N49 and N53 were known to be affected by the tawny rust all over the country. Fortunately, the disease was not reported from any other sugarcane growing countries (Rutherford 2018).

### The characteristics of rust symptoms

In general, the rust diseases appear on leaves and the stalks are free from attack. On leaves, the brown rust symptoms start with small, light green to yellow dots turn to narrow, elongated lesions on both leaf sides. The spots are surrounded by pale yellow-green halo turn to reddish brown. The lesions are 2-10 mm in length and sub epidermal uredinia develop on lower side of leaves, rupture the epidermis and release the uredospores. The lesions are formed parallel to mid rib of 2-6 months old plant leaves. In severe cases, lesions give the individual leaves a brown or rusty appearance and premature death of leaves result in a burnt appearance to the crop. The number of green leaves per plant, the cane length, diameter and number of canes per stool are also affected (Fig. 3a).





c. Tawny rust (courtesy: Sharon McFarlane, SASRI)

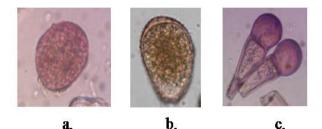
The orange rust symptoms are also same as of brown rust resulting confusion in early stage of infection. The symptom appears as very minute, elongated vellowish spots surrounded with pale yellow-green halo. The length of spot increases over a period of time but the matured lesion is shorter than other two rusts, oval shape, orange to orange brown or yellow brown in colour. The uredinia occur mainly on under surface of leaves and sometimes on upper side also in some varieties which are more than 6 months old (Fig. 3b). Depending on host susceptibility and environmental conditions the visible rust symptoms appear 8-18 days after the initial infection (Arya and Perello 2010). Orange rust pustules always occur in group and present in the lower half of the leaf. It occurs in hot humid weather in summer and in warm to cool humid conditions in autumn and appears during the late crop stage (Egan 1964).

The tawny rust attacks all the stages of the crop and favoured by cool and moist weather. The orange colour lesions are 2-10 mm linear and parallel to mid rib of leaves. The tawny rust lesions are 2-20 mm long, 1-3 mm wide and similar in size to brown rust (Fig. 3c). The pustules are produced on both sides of the leaf and the lesions are formed towards the leaf tip in severe form. The rust pustules release orange coloured uredospores on leaf surfaces once they mature.

# Spore morphology and biology of rust pathogens

All the three sugarcane rusts are autoecious in nature producing uredospores and teliospores on sugarcane only. In both brown and orange rusts, uredospores are more common in the field throughout the year. In case of P. melanocephala teliospores are formed towards the end of the season and teliospores of P. kuehnii were recorded only by Butler (1914). The role of basidiospores in disease cycle is not known on sugarcane and the alternate hosts for both brown and orange rust were not identified (Purdy et al. 1983). In case of M. fulva also the teliospores were not observed however, the appearance of tawny rust on Miscanthidium capense grass suggested the possibility of teliospores production under cold stress (Rutherford 2018).

Many research groups described the rusts based on uredospores morphology. *P. melanocephala* has small cinnamon to dark brown uredospores in the range of 25-39 x 17-28  $\mu$ m in size (Fig 4a). The uredospores of *P. melanocephala* are obovoid or ellipsoidal with uniform wall thickness of 0.8-2.3  $\mu$ m whereas, uredospores of *P. kuehnii* are orange to reddish brown colored and larger with size of 33-53 x 21-31  $\mu$ m (Fig 4b). The uredospores (25-32 x 20-26  $\mu$ m size) of *M. fulva* are bright orange to orange-red compared to brown and orange rusts. The uredospores of *P. melanocephala* 



**Fig. 4.** Morphological features of rust spores a. Uredospore of *P. melanocephala* b. Uredospore of *P. kuehnii* 

c. Telispores of *P. melanocephala* 

and *M. fulva* have uniform wall thickness but *P.* kuehnii has apically thickened walls. The presence of paraphyses in P. melanocephala and absent in P. kuehnii is the major distinguishing feature between the two species. However, the occurrence of intermediates makes the task of mycologists difficult to differentiate of orange or brown rusts based on morphology. The presence of spines, the spacing and densities of spines at the pore caps can be easily observed under light microscopy. In case of *P. melanocephala*, the spines are arranged at regular intervals of 1-1.5 µm spacing and at pore cap they are arranged in clusters whereas in P. kuehnii, the spines are arranged at 3-4 µm spacing and they are not a clustered at the pore top (Mordue 1985).

The telial stage was first observed on *S.* officinarum with the mean teliospore size of 51.0 x 26.4 µm and found infecting *S. officianarum* and not *Sorghum vulgare* (Patel et al. 1950). The teliospores were bigger in size than those obtained from *S. spontaneum* and the rust was named as *P. sacchari*. Only *P. melanocephala* produces two celled black to dark brown teliospores with darker upper cells (Fig. 4c) and is uncommon in *P. kuehnii* (Mordue 1985; Virtudazo et al. 2001a).

Uredospores germination and infection of *P. melanocephala* occur over a temperature range of 5-34°C but the optimal temperature range is 15-

25°C (Barrera et al. 2012). It was reported that there was 50% germination of urediniospores *in vitro* at temperatures between 20 and 29°C for both *P. melanocephala* and *P. kuehnii*. The uredospores of *P. melanocephala* germinated from 20 to 32°C and at 31°C, 40% of spores germinated and stopped germinating at 33°C. The germination of *P. kuehnii* uredospores dropped from 65% at 29°C to 18% at 30°C and was zero at 31°C (Sanjel et al. 2019).

The uredospores of tawny rust were found to germinate at 100 % relative humidity both under wet and dry conditions whereas the brown rust spores require free thin film of moisture on leaves (Martin et al. 2015). For tawny rust, the optimum temperature for uredospore germination was in between 18 and 22 °C and the maximum germination was observed at 19 °C when free moisture is available (Martin et al. 2015)..

Uredospores failed to germinate after 5 weeks in cool environment but lose viability very quickly in hot weather conditions. The germ tubes were unable to reach the stomata and cause infection below 15°C and above 30°C temperature (Egan 1964). Uredospores germinate only when water is present for at least 6h or nine hours of leaf wetness at > 98% relative humidity (Sotomayor et al.1983; Ramouthar 2009). It was observed that *P. melanocephala* was very active when the favourable environment prevailed with a 7-h leaf wetness period at 17°C but not with a 4-h period (Raid and Comstock 2000; Barrera et al. 2012).

# Variability of rust pathogens and physiological races

There are worldwide reports available on rust resistance breakdown in sugarcane and the existence of races in sugarcane rust pathogens (Shine et al. 2005). Through breeding programme, many varieties are being developed with multiple disease resistance in many countries. Though rust resistance is heritable (Comstock et al. 1992; Ramdoyal et al. 2000) the durability of rust resistance is broken down due to development of new rust races (Shine et al. 2005).

The earlier work on rust differentials was initiated in India during 1960s and detailed studies on rust races were carried out by several workers (Srinivasan and Muthaiyan 1965; Muthiyan et al. 1966). In the beginning, 43 varieties comprised of 27 hybrid canes, 8 S. officinarum. 7 S. barberi and one S. edule were utilized in screening of 32 rust isolates collected from Coimbatore, Cuddalore and Mysore infecting different varieties. Of these 43, varieties showing uniform resistant reactions against all the rust isolates were discarded and remaining 18 varieties viz., B 35197, B 35269, B 42279, B 4327, BO 24, Co 349, Co 419, Co 421, Co 527, Co 603, CP 29/116, CP 43/33, H 45-2708, H 49/5, POJ 2775, Baroukha, Basta Old and Dhaur were included for race studies along with universal rust susceptible variety Co 475. Using these differentials, 5 physiologic races viz., race 1-5 and one sub race of P. melanocephala were identified and among these, race 5 was present in the field during March to October which had high temperature tolerance of prevailing

29.4 °C and 21.1 °C maximum and minimum temperatures respectively at Coimbatore. Race 1 of *P. melanocephala* showed R to MR reactions on all differential varieties except Co 475 which showed S reaction. It has two sub groups and race IA and IB based on reaction on the cv Co 475. The outcomes of the early period rust research during 1970's at ICAR-SBI, on the rust reactions of physiologic race of *P. melanocephala* is summarized in the table 1.

Later, the differentials were reduced to 11 viz., B 35269, B 4327, Co 349, Co 419, Co 603, CP 29/116, CP 43/33, H 45-2708, H 49-5, Baroukha and Basta Old (Srinivasan and Muthaiyan 1965). A new race was identified while studying reactions of 3 cultures isolated from Co 62077, Co 349 and H 44-2818 on 11 differentials which is not of temperature tolerant like race 5 and appeared only during cooler months at Coimbatore (Muthaiyan et al. 1966). But in Australia, race diversity in brown rust could not be detected while using differential sets comprised of Co 419, Co 421, Co 475, Co 603, CP 29-116, CP 43-33, CP 44-101, H 49-5, 67N 1352, Q 87, Q 90, Q 110. After studying the rust reaction types of isolates collected from 1978-1986, it was concluded that there is only

Race	Brown rust reactions				
	R	MR	S	HS	Mesothetic
Race I A	B 35197	POJ 2775, Basta Old	Co 475		
Race I B	POJ 2775 and Basta Old	B 35197		Co 475	
Race 2		Co 349 and Basta Old	Co 419	Co 603	
Race 3	All other varieties		Co 419	Co 475	
Race 4	All other varieties	Co 421, Co 527, B 35197, BO 24, POJ 2775, H 45- 2708, Baroukha and Dhaur	H 49/5	B 4327	Co 475
Race 5	All other varieties	B 42279	Co 349, B 35269 and CP 29/116	H 49/5, H 45- 2708 and CP 43/33	

Table 1. Brown rust reactions on differential hosts by P. melanocephala races

one physiological race present in Queensland and the rust expression is influenced by favourable environment and not due to development of any new races (Taylor 1992). In Florida, prior to 1984, only two races were identified and since 2005, four races of P. melanocephala were found to be present using a set of differentials consisting of popular varieties viz., CP 72-1210, CP 78-1247, CP 74-2005, B4362, CL 41-223 and CL 73-239 (Dean and Purdy 1984; Shine et al. 2005). Pocovi et al. (2010) observed significant molecular diversity among P. melanocephala isolates collected from three regions using AFLP markers in Argentina. However, in Brazil the existence of races could not be found based on aggressiveness among the P. melanocephala isolates of different regions (Peixoto et al. 2014).

It is evident that if there is any change in variability of existing pathogen, there is a possibility of breakdown of resistance in popular varieties under cultivation (Taylor 1992). Virtudazo et al. (2001b) suggested that the relationships among Puccinia isolates can be clearly understood while studying the D1/D2 region sequences than comparing ITS regions. Dixon et al. (2010) could not find any close association between P. melanocephala and P. kuehnii while analyzing sequences of ITS and large subunit (nLSU) loci. Molecular analyses were conducted to identify the basis of new race evolution in P. kuehnii especially on the conserved rRNA repetitive elements in Australia. Braithwaite et al. (2009) studied internal transcribed spacer (ITS), IGS and LSU regions of rDNA among orange rust isolates from different locations in Australia and confirmed that a single mutation might have occurred in P. kuehnii to produce a virulent race on the cv Q124 causing orange rust epidemics during the year 2000.

Since both rusts appear on the same crop, there are chances for misidentification of two *Puccinia* spp. in the early stages of infection (Egan 1980; Virtudazo et al. 2001a). The identification of

uredospores through conventional method is cumbersome while observing the germinating spores or in early stages of infection. Therefore for accurate identification of the species from samples collected from leaf, soil and rain, molecular methods are useful where the inoculum load is very low. Glynn et al. (2010) developed species specific primers for detection of orange and brown rust pathogens at very early stages of infection or at low level of DNA concentration collected from spore or mycelia of the rust pathogens.

An easy, quicker cost effective assay for early disease detection of *P. kuehnii* was developed and in this loop-mediated isothermal amplification (LAMP)-based assay, under isothermal conditions, DNA is amplified with high specificity using four primers corresponding to a unique DNA sequence of *P. kuehnii*. In the positive samples, the final color change from orange to green upon SYBR Green I dye addition in the product without any post-amplification processes makes the method convenient (Chandra et al. 2016)

#### Epidemiology of rust and uredospores dispersal

Across the countries, a good progress has been made on brown rust epidemiology. The production of uredospores is influenced the temperature and relative humidity. It was noticed that rust spores present abundantly during the cool, windy and dry seasons. The maximum uredospore population was recorded during noon time and early afternoon (Tilak and Kulkarni 1978). Comstock and Ferreira (1986) observed that uredospores were produced more after heavy rain followed by bright sunlight. However, the spores were easily washed off due to heavy rain from the leaf surface thus reducing disease severity. Raid et al. (2013) observed that orange rust severity in Florida has a negative correlation with frost intensity during the winter months and the severe frost reduces the viability of rust spores. The maximum rust severity was observed in environments with 50% or more relative humidity and with minimum temperatures of 20°C or less during day time. Rainfall alone is not sufficient for expression of high rust severity (Comstock and Ferreira 1986). The severe winter in Texas and Louisiana hindered the activity of rust pathogens preventing the occurrence of epidemics (Comstock 1996).

Many grass weeds and other host plants were known to harbour rust pathogens thus serving as collateral hosts for the obligate pathogens (Virtudazo et al. 2001a). In addition, *P. melanocephala* was reported on *Erianthus ravennae*, *E. fulvus*, *E. rufipilus* and *Narenga porphyrocoma* L. (Autrey et al. 1996; Purdy et al. 1983; Raid and Comstock 2000).

The uredospores otherwise known as repeating spores, produced in larger quantity by the rust fungi with the higher dispersal potential are mainly responsible for rust epiphytotics (Nascimento et al. 2012). Uredospores with thickened cell wall and fine spines help the spores to be carried for a long distance by attaching themselves with human clothes, animals or any moving objects in the atmosphere (Wang et al. 2010). The spread of rust from a country to its neighbour has been attributed by the wind current carrying the rust spores and also step by step establishment of the pathogen in sugarcane areas over a period of time. Purdy et al. (1983) discarded the possibilities of P. melanocephala introduction to Dominican Republic through change in crop management practices or through infected setts or through workers. The transoceanic movement of rust inoculum from Cameroon was possible through wind during July 1978 and distributed in neighboring states. During the same period, introduction of brown rust in Queensland, Australia was observed and it was also attributed to monsoon winds across the Indian Ocean

affecting the major varieties in larger areas (Taylor 1992; Whittle and Holder 1980). Mims and Mims (2003) speculated that smoke generated through sugarcane trash burning in Cameroon would have resulted in transportation of brown rust uredospores to the Dominican Republic. However, Rutherford (2018) opined that the role of teliospores in dispersal process is very limited as they are produced at the end of crop season where green leaves become unavailable and the environment is not favourable for the pathogen.

## Seasonal appearance of rust

As in cereal rusts, the rust severity depends on the climatic conditions and the varietal susceptibility. The rusts show their presence on leaves depending on the temperature, leaf wetness, and stage of the crop as flecks to rust pustules rupturing of epidermis. The susceptible host genotype, favourable environment and inoculum load of pathogens are responsible for development of brown and orange rusts in sugarcane (Barrera et al. 2012; Comstock and Ferreira 1986; Irey 1987; Purdy et al. 1983; Raid and Comstock 2000; Sandoval et al. 1983). It was demonstrated that variety and environmental factors played a major role in expression of rust severity in Argentina. During the first four months, brown rust showed a rapid increase in damage index and subsequently decreased with increase in plant age (Victoria et al. 1989). However, the highest brown rust severity was observed on 4 - to 6 month old, and 5 to 7 month old plants in Hawaii, and Cuba, respectively (Comstock and Ferreira 1986; Sandoval et al. 1983).

Depending on the planting time in various countries and the climatic conditions, the time of rust appearance varies. In Australia, brown rust was noticed during August to December period where

the dew at night time is favourable (Magarey et al. 2004). However, the brown rust epidemic depends on the prevailing temperatures during winter and spring. In Florida, brown rust was most severe during mid-May to mid-July, whereas orange rust was severe at two intervals during mid-May to early August on 5-9 months old sugarcane and the second one was from November to December on 10 to 12 months old sugarcane indicating plant age is not a limiting factor in orange rust development. During 2014, the brown rust epidemic was reported in late March due to warmer environment favouring P. melanocephala (Sanjel et al. 2019). In Zimbabwe, brown rust was observed from May to August when mean temperature of 20 °C and 70-90% relative humidity (Zuoutete 2006). In the Dominican republic, the brown rust was observed during December to February when temperature range was 15-30 °C and the humidity was 70-73% (Bernard 1980b). In India, under Maharashtra conditions, rust was observed during November to February and more severe in January, when there was 30.1°C temperature and 57.3% relative humidity (Lambhate et al. 1976). A bimodal disease progress of rust was observed in the field while analyzing 10 years weather data of 2007-2017 at ICAR-SBI in peninsular India and the disease severity maxima was obtained during July-August and November to January (Selvakumar and Viswanathan 2017).

During 2016, brown rust was observed in October planted sugarcane variety Co 0403 susceptible to rust in the month of January where the maximum temperature was 31.7 °C and minimum temperature at 19.3 °C. There was a steady increase of rust severity from 15% in January to 20% in March and then there was rapid fall in rust severity from April to September. The brown rust was noticed in February planted crop of the cv Co 0403 during July (5 months old) as pustules and 10% severity was noticed during September 2016. The severity increased to 20% during November and the severity was maintained at the same level till harvest of the crop (Selvakumar and Viswanathan 2017).

The changes in day and night temperatures and erratic rainfall under changing climatic conditions may influence the severity of sugarcane rust from minor to severe on some varieties. Although climate change issues are not clearly felt under Indian scenario, recently we witnessed sudden occurrences of severe rust on the varieties like CoVSI 7805 and CoVc 03165 in the states of Karnataka and Maharashtra (Viswanathan 2012, 2013). High yielding varieties Co 0218 and Co 0403 developed at ICAR-SBI, Coimbatore could not be popularized due to their susceptible nature to rust under field conditions. Although these varieties undergone long breeding cycle, during the evaluation stages their rust susceptibility could not be ascertained in natural conditions due to small size plots.

#### **Breeding for rust resistance**

As rusts are strictly air borne, there is a great concern that the disease will spread to the new sugarcane growing areas rapidly. Due to genetic variability in rust pathogens, many excellent commercial varieties become susceptible and even resistant clones tend to become susceptible over a period of time (Braithwaite et al. 2009). Among the three rusts of sugarcane, brown rust pathogen P. melanocephala has high adaptability to the changing climate and high potential to overcome the resistance offered by single dominant gene (Hoy et al. 2014). Therefore, there is a need to identify the sources of rust resistance in parental clones used in crossing programme and the presence of diverse genes for resistance in cane germplasm throughout the world.

#### Screening for rust resistance

Identification of rust resistant lines in the field is cumbersome as the disease development is highly influenced by the environment. Various methods and scales have been standardized for screening for rusts resistance in the field. Liu (1980) in Puerto Rico, evaluated the commercial varieties and promising lines for brown rust resistance under artificial inoculations. Wrapping the young shoot with rust affected leaves was found to be better than spraying and dusting uredospores. In Florida, leaf whorl inoculation technique was standardized and uredospores (a) 10<sup>5</sup> per ml in whorls were introduced and symptoms were observed 30 days after inoculation at weekly intervals. The disease rating scale of 0-4 was followed where 0 and 1 were resistant and 2 was moderately resistant and 3 and 4 were considered as susceptible. Following the criteria, the sugarcane clones are screened for orange rust resistance following leaf whorl inoculation technique under field conditions (Sood et al. 2009, 2013). In China, Wang et al. (2013) screened the germplasm after spraying the field with uredospores suspension and observed for symptoms after 4-5 weeks using 0-9 scale. Under All India Coordinated Research project on sugarcane detailed experiments were conducted at Padegaon, Pune, Sankeswar and Anakapalle to standardize a methodology to screen zonal varieties for rust resistance. Among the two inoculation methods viz., clip inoculation in the leaf whorl and leaf whorl inoculation revealed that the latter was found effective in causing more severe reaction on a set of susceptible varieties and ideal to screen the clones for rust resistance (Viswanathan, unpublished). In Peninsular India, screening of 275 sugarcane parental clones for rust resistance under natural conditions at Coimbatore revealed that ~60% of clones remained free from rust and 20% exhibited moderately resistant (MR) reactions. Of the 20% susceptible group, ~13%

were moderately susceptible (MS) and 7% were susceptible (Selvakumar et al. 2018).

# Use of molecular markers for Bru genes identification

Sugarcane rust resistance is controlled by one or a few major genes or in some cases minor genes imparting rust resistance (Asnaghi et al. 2001; Ramdoyal et al. 2000). Identification and development of rust resistant material will take many years hence it is suggested that marker assisted selection can reduce the length of the breeding cycle and increase selection efficiency with more precision (Miedaner and Korzun, 2012). Two major brown rust resistance genes, Brul and Bru2 were identified in sugarcane (Daugrois et al. 1996; Raboin et al. 2006). Brul is the first well characterized Mendelian gene in sugarcane and is found to be effective against P. melanocephala across countries and the markers R12H16 and 9020-F4 were developed for detection of Brul. This Brul gene was identified in the cultivar R570, the most popular brown rust resistant cultivar in Reunion island and other places, cultivated for more than 20 years (Asnaghi et al. 2004). Two SNPs for Brul A1 and Brul A2 were identified and unlabeled probe assay was designed in a closed tube to target these SNPs. This probe can be effectively utilized for rapid detection of Brul locus with high cost reduction (McCord and Migneault 2016).

It is reported that brown rust resistance in the current sugarcane varieties mainly relies on the *Bru1* gene (Costet et al. 2012). In Guatemala, *Bru1* was reported in more than 32% of 80 varieties (Molina et al. 2013). Glynn et al. (2013) identified *Bru1* genes responsible for brown rust resistance in 32% of 485 parental clones in Florida whereas in Argentina, 49 accessions out of 129 were found to be brown rust resistant but only 16% carry *Bru1* 

gene indicating involvement of alternate genes for brown rust resistance (Racedo et al. 2013).

#### Rust management through chemicals

Though development of resistant varieties is the viable option to control rusts of sugarcane, in severe epidemic condition, usage of chemicals is recommended on larger areas as an alternative control. Many fungicide groups are available which can effectively control the rusts in the field. The fungicides may be of protective, curative, eradicant or as antisporulant. The ergosterol synthesis inhibitors such as triazoles have less effect on urdospore germination since the germ tube can grow even in the absence of sterol (Mueller et al. 2004). The strobilurin and carboximide group of fungicides affect the various enzymes involved in mitochondrial respiratory chain of fungi disrupting the energy production during spore germination, host penetration and sporulation (Buck and Williams 2003). However, azoxystrobin group act as protective, curative as well as antisporulating fungicides make them a very effective rust controlling fungicides (Mueller et al. 2004). The efficacy of fungicides viz., Pyraclostrobin (Headline®), fluxapyroxad + pyraclostrobin (Priaxor®) and azoxystrobin + propiconazole (Quilt Xcel®) were tested in detached leaf assay against urediniospore germination of both P. kuehnii and *P. melanocephala* and were found effective at 0.01, 0.1, and 1 µg/ml. Whereas triazole (metconazole) and carboxamide (fluxapyroxad) fungicides were highly effective in reducing spore germination only at higher concentrations @ 10 µg/ml. Further it was observed that P. melanocephala is highly sensitive than P. kuehnii against the same concentration of fungicides metconazole and fluxapyroxad (Chaulagain et al. 2019).

The number of fungicide sprays depend on severity of rust at two weeks intervals (Bernard and Liu

1980; Hoy and Savario 2007; Jiang, 1985; Raid and Comstock 2013; Staier et al. 2003; Taylor et al. 1986; Zvoutete, 2006). In Florida, spraving of fluxapyroxad + pyraclostrobin fungicide at three week-intervals on a popular variety CP 96-1252 during early, mid, and late season in the brown rust infected field resulted in 40-42% less rust severity than the untreated control. The fungicides also helped in 27-35% increase in mean stalk weight and minimum of two spraving are recommended for effective control of brown rust in Florida (Chaulagain et al. 2019). The fungicide application at the initial time of orange rust appearance resulted in the lowest rust severity and development throughout the season and enhanced mean stalk weight. Spraying of fungicides with different modes of action is recommended to avoid build up of fungicide tolerant races. In a field, two consecutive sprayings of fluxapyroxad +pyraclostrobin followed by one application of metconazole on an orange rust susceptible variety CL85-1040 grown in Florida resulted in same results similar to another set of fungicides with different mode of action such as fluxapyroxad + pyraclostrobin, fluxapyroxad, and pyraclostrobin during three consecutive months in controlling rust development (Chaulagain et al. 2019c).

Although effective chemicals are available, fungicide sprays in a grown up crop pose practical difficulties. Since the occurrence of rust is highly dependent on the environment, growing of rust resistant varieties and modification of planting time are the general recommendations in Zimbabwe (Zuoutete 2006). The need for frequent fungicide applications and the low net profit obtained after control restricted the use of fungicides against rust in Florida (Zhao et al. 2015).

#### **Future trends**

Development of resistant varieties is the most viable, sustainable and environment-friendly

disease management strategy and this has been a high priority for breeding programmes. Indian sugarcane breeding programme is guided by the red rot and smut resistance along with the yield and quality of the progenies (Hemaprabha et al. 2018). The requirement for rust resistance exerts an additional burden on the selection process of superior lines in breeding programme in a country like India. The available parental clones need to be screened under controlled or artificially inoculated conditions to identify sources of rust resistance. There is a need to study the contribution of Brul in rust resistance among the parental clones in India. The genetics of rust resistance genes in the donors need to be known for gene pyramiding to achieve a high level of durable field resistance. Similar to Bru1 gene marker, there is a need to develop markers for identifying the orange rust resistance gene. In addition, synteny between brown rust and orange rust resistance in the parents and progenies is to be established. Since the pathogens have transoceanic movement, there is a need to study variability in rusts on a global scale. The identification of race frequency and distribution in a continent will further help breeders and pathologists to keep a vigil on development of any new races or introduction from any other sources. Frequent monitoring of the disease will help in tracking the movement and establishment of rust pathogens in new areas.

Focused study on the interaction of leaf wetness, relative humidity, time of penetration, and stage of the crop in epidemiological aspects would lead to development of tangible rust prediction models. In a nutshell, development of clones with agronomically superior traits coupled with resistant genes will yield fruitful results to the stakeholders. The reduction in area of susceptible varieties will restrict the spread of air borne rust diseases. The mosaic pattern of rust resistance in the field will help in minimizing the loss due to the rust introduction into new areas.

### Conclusion

Over the decades, sugarcane rusts spread throughout the world and often posed as a serious disease in different countries. Orange rust of sugarcane is a classical example of minor disease becoming a major disease under changing climatic conditions. Further, rusts are a growing concern to cane cultivation due to adoption of a single variety on larger areas in a favourable environment. The emergence of rust in recent years has tuned the breeding strategy worldwide to incorporate rust resistance in the upcoming new varieties. The effective method of rust inoculation and screening methods were standardized in many countries and the rust resistant sources were identified. In Australia, China and USA, the available molecular markers for Bru 1 gene were utilized in identification of rust resistant sources. Many sources for alternate resistant genes have also been identified and are being used as parents in crossing programme. The molecular markers were identified to detect the variability of rust pathogens in few countries. Effective fungicides were identified to control the rusts before the disease outbreak. The development of rust prediction models is underway with the information on critical weather parameters such as optimum temperature, relative humidity and leaf wetness duration on rust infection. Research efforts made in different countries could contain the threats posed by the rusts and severe economic losses were averted. Under Indian scenario, cost of cultivation increases in sugarcane owing to various factors. Hence, there is a need to tackle rust through host resistance to sustain sugarcane cultivation and increased cane productivity and the losses caused by the rusts and other minor diseases have to be addressed through a systematic approach.

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