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Inheritance pattern of cold tolerance in pigeonpea [*Cajanus cajan* (L.) Millsp.]

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Abstract

Among all the abiotic stresses, cold is one of the important factor limiting crop productivity. Medium and late maturing varieties cover most of the cultivated area of pigeonpea and therefore the chances of facing cold/frost are prominently high. Northern parts of the country are witnessing very low temperature during the last fort night of December and first fortnight of January, which is conducive for frost injury and the susceptible lines exhibit frost symptoms. Screening of 302 germplasm/lines of pigeonpea comprising of varieties and advance materials against frost injury during the years 2016-2018 facilitated to identify tolerant and susceptible lines. One hundred and forty one lines did not show any injury symptom, whereas 120 were classified under score 1 and 17 lines showed moderate symptoms to frost injury. A highly frost tolerant (insensitive) line ICP 10509 was crossed to susceptible (sensitive) line ICP 11182 to study the nature of frost injury and mode of inheritance. The F_1 hybrid showed tolerance to frost injury with 1-2 leaves showing little symptom (score 0-1) indicating dominance of the trait. The F_2 population segregated into tolerant (216 plants) and susceptible individuals (91 plants) fit well into expected ratio of 3(T): 1(S) (P value = 0.06027) signifying that frost tolerance (insensitivity) is controlled by a single dominant gene. The proposed hypothesis was verified by backcross populations; B_1 (F_1 x ICP 11182) segregating into 1(T):1(S) ratio (P value = 0.1237), whereas, all the plants in B_2 (F_1 x ICP 10509) generation exhibited tolerance to frost injury. The identification of a single gene exhibiting tolerance to frost injury may be useful for developing frost tolerant genotypes.

Key words: Cold tolerance, frost injury, temperature, screening, inheritance

Introduction

Among the pulse crops, pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important source of protein in a country like India, where majority of population is vegetarian. This crop is grown in semi-arid regions of the world including India. The maximum area under pigeonpea cultivation in India lies in Maharashtra, Karnataka, Andhra Pradesh, Madhya Pradesh, Uttar Pradesh and Gujarat. Historically, pigeonpea has been cultivated on less productive and marginal soil with minimal inputs and grown mostly as an intercrop with other *kharif* crops under rainfed ecology. Since the pigeonpea is a widely adapted long duration crop, it is exposed to wet, cold and dry weather conditions. The frost injury is also caused in other cereal and pulse crops and an important limiting factor for pulse production worldwide including India (Maqbool et al. 2009). Generally, the radiant frost damage in *Phaseolus* species is caused by a nocturnal net loss of long wave radiations, causing the plant to cool to temperature subsequently below ambient temperature (Balasubramanium et al. 2004). Legume crops including field pea, faba bean, chickpea and lentil are very sensitive to chilling and freezing temperatures particularly at the time of flowering, early pod formation and seed filling stages. The frost damage also occurs in other cereal, vegetable and fruit crops (Malhotra and Saxena 1993; Wang 1987). Low and freezing temperatures causes flower sterility, poor dehiscence of pollen mainly due to dense chilled air settles around the plant foliage and flower bunch where the most damage occurs. The cold air is likely to cause nucleation of the intracellular fluid in plant tissues and the subsequent rupturing of the pollen membrane (Muqbool et al. 2009; Balasubramanium et al. 2004). Also during cold acclimation the rate of photosynthesis

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is decreased resulting in reduced plant growth, reduced water content in tissues and the accumulation of solutes (Margesin et al. 2007). The chilling and freezing range temperatures are one of the three most important abiotic stresses (frost, heat stress and drought) causing flower sterility and pod abortion (Singh and Jana 1993). One of the first studies on cold injury in chickpea was conducted under field conditions at different temporal locations in India, and the results demonstrated differences in flower abscission percentages at different temperatures (Savithri et al. 1980). In northern and north eastern plains, the available long duration (late) varieties (maturity >200 days) are highly thermo-sensitive which are exposed to low and freezing (<3^{o}C) temperature during late vegetative and flowering stage and high (>35^{o}C) temperatures during pod setting stages resulting into considerable damage to foliage and pods leading to poor yield. Yong et al. (2002) reported that certain varieties of pigeonpea are highly sensitive to frost damage. However, limited information on frost injury in pigeonpea is available in literature and therefore, the information on cold tolerance in pigeonpea is necessary to develop cold tolerant genotypes. Tolerance to cold temperature depends on multiple factors like range of decrease in temperature, duration of low temperature, type of the genotype, age of plant, soil moisture etc. Out of these factors, temperature is playing more crucial role in pod setting and for even floral abortion (Turnbull et al. 1981; Omanga et al. 1995; Nayyar et al. 2005). Considering the potential of damage by frost, a study was conducted to screen a large number of pigeonpea germplasm lines and to understand the nature and magnitude of damage caused by freezing temperatures. The study on to determine the mode of inheritance of cold tolerance was also conducted.

Materials and methods

A total of 302 lines comprising of germplasm, varieties and advance materials were screened for cold tolerance for two years during the years 2017-18 and 2018-19. The germplasm lines were sown in rows of 3m length 75 cm apart with recommended plant to plant distance in the *kharif* seasons of 2016 and 2017. However, the parental materials were also screened during 2016-17. The plants were scored for frost injury on five-point scale (Yong et al. 2002) with little modification as: 0 = Tolerant, no visible symptom of damage; 1 = tolerant up to 10% leaves killed; 2 = moderately tolerant only terminal branches and tender leaves killed; 3 = Moderately susceptible, upper-half of the plant canopy killed; and 4 = susceptible, all the leaves are affected rather killed. The genotypes were classified according to the maximum score and tabulated. A tolerant line ICP 10509 (Fig. 1) was crossed to susceptible genotype ICP 11182 (Fig. 2) in 2016 and the F_1 was grown in 2017. The F_1 hybrid was selfed and crossed to both the parents to obtain F_2 and backcross generation. The parents, F_2 (307 plants), B_1 (F_1 x ICP 11182, 51 plants) and B_2 (F_1 x ICP 10509, 28 plants) generation were planted in *kharif* 2018 and the frost injury was recorded in each plant during the coolest period in month of December-January when temperatures were freezing and conducive. The minimum and maximum temperatures recorded during crop seasons in 2016-17, 2017=18 and 2018-19 is presented graphically in Figs. 3A, 3B and 3C. The plants were classified accordingly after scoring the frost damage. The plants exhibiting 0 to 1 score were merged and considered as tolerant while the individuals with 3-4 score were considered as susceptible. Chi-square test was applied to test the goodness of fit.

Results and discussion

The germplasm lines were evaluated against frost injury in unreplicated trial over three years. The minimum and maximum temperatures during November to January in each year (2016-17, 2017-18 and 2018-19) were recorded daily to correlate frost injury with the prevailing temperatures of the coolest period. The range of temperature (minimum and maximum) from November to January for each year is depicted by graphical representation in Fig. 3. Low temperatures were recorded in more than one spell during the winter season in each year. The minimum temperature recorded was 1^{o}C on 14th January 2017 and prevailed over 4 days (1^{o}C to 1.5^{o}C). The minimum temperature on 29th December 2018 recorded was 0.5^{o}C with average temperature of 2.3^{o}C during 17th December 2018 to 1st January 2019. During the second spell of coolest period from 5th January 2019 to 22nd January 2019 the lowest temperature recorded was 1.1^{o}C with an average temperature of 3.5^{o}C. The maximum damage by frost injury occurred during the month of December-January under which the genotypes were scored on 0-4 scale. Distinct varietal differences in response to low temperatures were observed among the genotypes. The tolerant genotypes suffered least to the frost injury while the leaves, flower buds and pods were damaged in susceptible plants (Figs. 1 and 2). Out of 302 germplasm lines (Table 1), 261 showed tolerance to frost with the score of 0-1 and the remaining

Fig. 1. A field view of parental line ICP 10509 showing resistance to frost

Fig. 2. A field view of parental line ICP 11182 showing susceptibility to frost. Arrows indicating the injury to flower buds, pods and leaves caused by frost

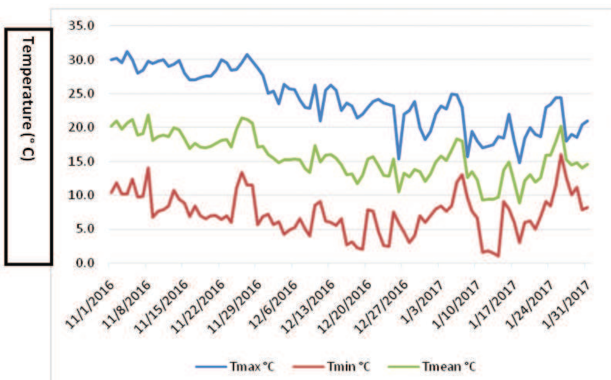

Fig. 3A. Temperature variation during November 2016 to January 2017

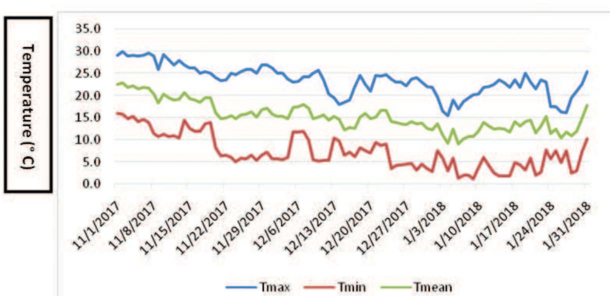

Fig. 3B. Temperature variation during November 2017 to January 2018

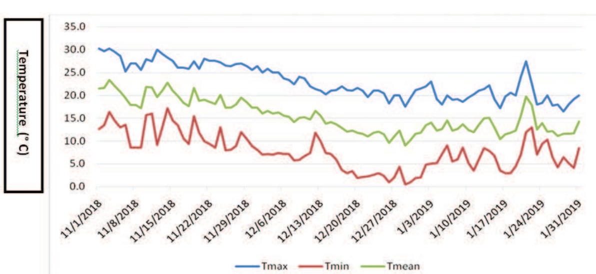

Fig. 3C. Temperature variation during November 2018 to January 2019

Fig. 4a. Left: The leaves of P_1, ICP 10509 (Resistant); Middle: The leaves of F_1 hybrid and Right: The leaves of P_2, ICP 11182 with injury symptom

Fig. 4b. Left: The foliage with flower buds of P_1, ICP 10509 (Resistant); Middle: The foliage, flower and pods of F_1 hybrid (Tolerant) and Right: The foliage and flower buds of P_2, ICP 11182 (Susceptible)

41 genotypes showed susceptibility to frost and showed injury of the foliage (>20 % foliage damage). Unlike cereal crops, it is difficult to analyse and score frost damage in grain legumes due to presence of various phenotypes on one plant at the reproductive stage particularly in pigeonpea due to its indeterminate habit. During the frost events minimum plant temperature of wheat can be typically ~1 to 3°C colder

Table 1. Resistant and susceptible germplasm lines categorized based on 0-4 scale against frost injury during three years

Scale				
0	1	2	3	4
BSMR 735, ICP 10509, ICP 10094, ICP 10397, ICP 10447, ICP 10503, ICP 10559, ICP 10654, ICP 1126, ICP 11281, ICP 11320, ICP 11321, ICP 11477, ICP 11946, ICP 12410, ICP 12515, ICP 12596, ICP 12654, ICP 1273, ICP 1279, ICP 12799, ICP 12842, ICP 13126, ICP 14094, ICP 14104, ICP 14116, ICP 14120, ICP 14147, ICP 14155, ICP 14229, ICP 14294, ICP 14368, ICP 14459, ICP 14471, ICP 14480, ICP 14547, ICP 14569, ICP 14638, ICP 14701, ICP 14722, ICP 14801, ICP 14819, ICP 14832, ICP 15452, ICP 15493, ICP 15511, ICP 15530, ICP 15629, ICP 15630, ICP 15922, , ICPL 20176, ICPL 20202, ICPL 20205, ICPL 20325, ICPL 20329, ICPL 20340, ICPL 24331, ICPL 2671, MAL- 6, PUSA 992, ICPA 2078 ICPB 2047, ICPH 3762, TJT 501, ICP 6973 , ICP 6974, ICP 14419, ICP 14416, ICP 14417, ICP 14907, ICP 15135, ICP 14019, ICP 12016, ICP 6450, ICP 6536, ICP 12017, ICP 13923, ICP 10897I, , ICP 15667, ICP 15761, ICP 15913, ICP 16264, ICP 16309, ICP 6370, ICP 655, ICP 6668, ICP 6739, ICP 772, ICP 7803, ICP 7869, ICP 7890, ICP 8793, ICP 8840, ICP 8860, , ICP 8921, ICP 8949, ICP 4715, ICP 4902, ICP 5142, ICP 5863, ICP 6049, ICP 6123, ICP 6128, ICP 7, ICP 7223, ICP 7260, ICP 7375, ICP 7426, ICP 7507, ICP 8012, ICP 8152, ICP 8227, ICP 10228, ICP 1071, ICP 11015, ICP 11059, ICP 11230, ICP 11543, ICP 1156, ICP 11627, ICP 12105, ICP 12123, ICP 12142, ICP 1216, ICP 12298, ICP 12358, ICP 8255, ICP 8602, ICP 8700, ICP 8757, ICP 9045, ICP 9750, ICP 995, ICP 13828, ICPL 11811, ICPL 12057, ICPL 12320, ICPL 13092, ICPL 20104, ICPL 20107, ICPL 20124	ICP 14885, ICP 14886, ICP 14900, ICP 14903, ICP 14952, ICP 14976, ICP 14976, ICP 15, ICP 15049, ICP 15068, ICP 15109, ICP 15142, ICP 15161, ICP 15185, ICP 15382, ICP 2577, ICP 2698, ICP 2746, ICP 3046, ICP 3049, ICP 3451, ICP 348, ICP 3576, ICP 38, ICP 4029, ICP 4167, ICP 4307, ICP 4317, ICP 4392, ICP 4575, ICP 13139, ICP 13241, ICP 13244, ICP 13270, ICP 13271, ICP 13304, ICP 13359, ICP 13431, ICP 13438, ICP 13571, ICP 13579, ICP 13633, ICP 13662, ICP 13852, ICP 13884, ICP 12825, ICP 13144, ICP 13618, ICP 6523, ICP 10906, ICP 6524, ICP 6527, ICP 14553, ICP 12029, ICP 13320, ICP 12733, ICP 12031, ICP 9336, ICP 939, ICP 9414, ICP 9655. ICP 9691, ICPL 10650, ICPL 11255, ICPL 11376, ICPL 11516, ICPL 131, ICPL 14282, ICPL 20094, ICPL 20096, ICP 10391, ICP 1508, ICP 10331, ICP 13562, ICP 13635, ICP 13283, ICP 13396, ICP 14969, ICP 110, ICP 6325, ICP 13193, ICP 6105, ICP 14594, ICP 10908, ICP 9510, ICP 14163, ICPL 20098, ICPL 20130, ICPL 2740, ICPL 281, ICPL 332, ICPL 7035, ICPL 8094, ICPL 87051, ICPL 87250, ICPL 88039, ICPL 96061, ICPL 99008, ICPL 99010, ICPL 99011, ICPL 99046, ICPL 99048, ICPL 469, Richa 2000, ICP 6817, ICP 8283, ICP 11990, ICP 13011, ICP 13103, ICP 13575, ICP 13577, ICP 15662, ICP 6815, ICP 6845, ICP 6859, ICP 7057, ICP 7076, ICP 7221, ICP 6929, ICP 6971	ICP 6992, ICP 7148, ICP 7314, ICP 7366, ICP 8266, ICP 11910, ICPL 14459, ICPL 20092, ICPL 20338, ICPL 11445, ICPB 2078, ICP 16335, ICP 7574, ICP 7101, MAL 13, A S H A , BSMR 736	ICP 8384, ICP 11823, ICP 11833, ICP 13167, ICPL 161, ICPL 85063, ICPL 99009, ICPL 87091, ICP 12680, ICP 13191, ICPL 15042, ICPL 15058, ICPL 96058, ICP 14444, ICP 6817, ICP 14802	ICP 11182, ICPL 151, Mutant Mal. 13, ICP 10908, ICPL 87, ICP 14923, ICPL 87154, I C P 8863

than canopy air temperature measured by exposed temperature probes (Fredericks et al. 2015) however, this is relatively poorly studied. Frost damage to different cereal crops can cause large yield penalties of 10-90% (Zhang et al. 2015; Frederiks et al. 2015) and is a significant limitation to grain production globally. To account for the damage caused by successive frost, Martino and Abbate (2019) recently

Table 2. Segregation of frost tolerant and susceptible plants in F_2, B_1 and B_2 generations in pigeonpea crosses

	Tolerant	Susceptible	Expected ratio	Chi- square	P value
ICP 10509 (P1)	98	0			
ICP 11182 (P2)	0	115			
F_1 (ICP 10509 x ICP 11182)	36	0			
F_2	216	91	3:1	3.53	0.06027
B1 (F_1x ICP 11182)	20	33	1:1	2.37	0.1237
B2 (F_1x ICP 10509)	51	0		51	

proposed a model using heat transfer in the wheat field. The extent of damage depends on phenophases on a particular plant (Maqbool et al. 2009). This is a typical case in pigeonpea and therefore, it is difficult to assess the damage caused by frost injury.

One of the first studies on cold injury in chickpea was conducted under field conditions at different temporal locations in India, and the results demonstrated differences in flower abscission percentages at different temperatures (Savithri et al. 1980). However, the high level of floral abortion and failure to set pod and seed in chickpea exposed to temperatures below 10°C showed considerable genetic variation for cold tolerance amongst the lines, even with the problems of field screening. The physiological basis of this variation during the early reproductive growth of chickpea indicates substantial genotypic variation in anther dehiscence and pollen viability (Srinivasan et al. 1999), but the study did not address freezing range temperature effects or tolerance.

The foliage in F_1 hybrid (ICP 10509 x ICP 11182) showed tolerance to low temperature (frost) but with little symptom of frost injury in one or two leaves indicating that the cold tolerance is dominant over susceptibility (Figs. 4a and 4b). In the F_2 generation 216 plants were categorized as frost tolerant (resistant) as they exhibited complete tolerance (0-1 score) and frost injury up to 10% leaves in few segregants, while 91 individuals recorded frost damage of the magnitude of >10% foliage damage (score 2-4). The frequency of F_2 segregants displaying tolerance (216 plants) and susceptibility (91 plants) fit well into the expected ratio of 3(T): 1 (S) indicating that tolerance to frost injury is controlled by a single dominant gene. The monogenic inheritance was confirmed by the data recorded in B_1 and B_2 generations. The leaves of 20 plants in B_1 (F_1 x ICP 11182) did not show any symptom of frost injury while 31 plants produced symptoms of frost injury. The observed data fit well into 1(T):1(S) ratio validating the F_2 hypothesis that the cold tolerance is determined by a single dominant gene. The B_2 population derived from the cross F_1 x ICP 10509 did not show any symptom of frost injury further confirming the expected theoretical ratio obtained in F_2 generation that the resistance to cold injury is indeed determined by a single dominant gene. However, the poor fit of chi-square value is likely due to some segregants might have escaped causing distortion in genetic ratio because the frost incidence is a natural phenomenon and the experiment is not conducted under artificial conditions.

Winter pulse crops are quite sensitive to frost injury and suffer marginal to high grain damage. The effects of the freezing temperatures also vary due to the nature of the crop. It is difficult to assess the amount of frost damage in pigeonpea because occurrence of frost is unpredictable and not a continuous phenomenon. Frost research to improve genetics or management solutions requires a robust experimental design that minimizes the effect of all variables that can cause plant damage except for the treatment (frost). Stutsel et al. (2019) suggested a design and proposed a prototype treatment of hot environment created by plot heaters around the field and monitoring the canopy temperature and air temperature during frost event showing that these remain above zero in the heated plots when ambient temperature drops below zero. The damage due to frost injury is related to flowering time and pod formation mainly due to frost resistance in *Pisum sativum* (Lejeune-He´naut et al. 1999, 2004). A gene (Hr) characterizing delayed flowering co-segregated with the most important quantitative trait locus (QTL) for frost resistance in European winter *P. sativum* material. The QTL related to frost stress in peas was identified using research findings in model species such as *Medicago truncatula* and *Lotus japonicus* (Stoddard et al. (2006). He advocated that modern genomic and molecular techniques can be applied to produce and breed frost tolerant genotypes. Earlier, Eujayl et al. (1999) identified one gene conferring resistance to radiant frost and found a randomly amplified polymorphic DNA marker that co-segregated with this gene at 9.1 cM. This suggests that late-flowering pea genotypes may be more likely to have frost tolerance. Certain varieties flower late and may escape the time of freezing temperatures. However, some varieties which have longer period of flowering and pod formation because of their indeterminate nature may caught up in low temperatures. In one of the study conducted on quantitative trait locus (QTL) mapping for winter hardiness in lentil indicated that tolerance to low temperature is a multi-genic trait (Kahraman et al. 2004). However, Eujayl et al. (1999) studied inheritance of radiation frost tolerance genes reported that this trait is believed to be controlled monogenically. These results are in contradicting those of Kahraman et al. (2004) who reported some QTLs for winter hardiness and showed that several genes control this trait. In chickpea, the frost injury causes flower sterility and pod abortion (Singh and Jana 1993). In 1991, Malhotra and Singh indicated that the genetic basis for cold tolerance in chickpea was dominant over cold sensitivity, and they also uncovered significant additive and non-additive gene effects.

Through this study, only limited information on nature of frost injury in pigeonpea is gathered. However, to determine the inheritance of frost injury (damage under low temperature) depends on multiple factors like range of decrease in temperature, duration of low temperature, genotype, age of plant, soil moisture etc. Therefore, it is bit difficult to assess the amount of frost damage in a crop because of its patchy nature and difficult in predicting compensation that may occur during grain filling (Yong et al. 2002) and to determine the exact damage caused by decrease in temperature alone and consequently the breeding for frost tolerance may also be cumbersome. With the availability of information on genome sequence (Singh et al. 2012; Varshney et al. 2012) in pigeonpea MAS can be adopted to breed frost tolerant genotypes. Also, in such a complex trait, a breeder must be able to screen a huge number of lines to select a progenies having all the desirable alleles.

Identification of a large number of tolerant genotypes indicated that pigeonpea is a unique plant with its ability to withstand various stresses. The recovery of new leaves occurred due to the perennial nature of the plant and the deep root system helps in maintaining the required optimum growth for plant survival. However, systematic experiments under controlled conditions are necessary to fully understand various aspect of frost injury and to assess the damage in pigionpea due to freezing temperatures.

Authors' contribution

Conceptualization of research (KD); Designing of the experiments (KD, RJ); Contribution of experimental materials (KD); Execution of field/lab experiments and data collection (KD, RJ, KK); Analysis of data and interpretation (KD, RJ, KK, RSJ, KG, RG); Preparation of manuscript (KD, RJ, KK).

Declaration

The authors declare no conflict of interest.

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