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Performance of Pigeon Pea [*Cajanus cajan* (L.) Millsp] Genotypes under Cold Stress

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ABSTRACT

Background: In north India, pigeon pea experiences low temperature stress during winter months (December-January). Information's regarding cold stress and its impact on morphological characters, physio-biochemical processes and yield attributes in pigeon pea crop is very limited. Keeping the above facts in to consideration, the experiment was formulated to screen suitable genotypes of pigeon pea tolerant to cold stress based on morpho-physiological traits and ability to set flower and pods and yield performance.

Methods: Field experiments with 98 genotypes of pigeon pea were conducted in augmented design including checks with all recommended cultural practices in 2018-19 and 2019-20 at New Research Farm, ICAR-IIPR, Kanpur. Morphological, biochemical and yield parameters were recoded periodically.

Result: Based on the reaction of pigeon pea genotypes against the exposer of cold stress in terms of flower/pod drop and flower/pod retention, cold injury at the apical meristems and post regeneration of apical portion, the genotypes like IPA 15F, Dhule-D, JBT46/27, IPACT-6, IPACT-14, IPAC-1-17, IPACT-68, IPACT-22 showed >90% flower/pod drop and retained only 0-10% flowers/pods, >90% apical damage and <10% recovery of apical portion and were considered highly sensitive to cold stress. Genotypes namely MA-3, IPA 9F, ICP 15-9-1, VKG 27/161, H-26, ICPL 7035, LRG-30, PBT/SSL 2/73, KPL 1034-31, IPA 15-15, KPL 34, ICP 2073, IPACT-5, IPAB 10-66, IPACT-10, IPACT-11, IPAHT-26, IPACT-15, IPAD 2-8, IPACT-16, IPAD-8, IPACT-17, IPAHT-43, IPACT-21 showed >80% flower/pod drop and retained 10-20% flower/pods, >80% apical damage and <20% recovery of apical portion and considered moderately susceptible to cold stress. Genotypes NDA-2, MAL-13, ICP-2275, IPACT-2 showed around 20-40% flower/pod drop and have retained 60-80% flowers/pods, <20% apical damage and >80% recovery of apical portion were considered as the highly tolerant to cold stress. The nitrogen balance index, chlorophyll and flavonols showed higher values in tolerant group whereas lower in susceptible group. The average yield/plant of pigeon pea genotypes tolerant to cold stress is considerably higher than the susceptible group of pigeon pea genotypes.

Key words: Cold stress, Cold tolerance, Cold susceptible, Flower/pod drop, Flower/pod retention, Genotypes, Pigeon pea, Reproductive capacity.

INTRODUCTION

In India, area occupied by pigeon pea is about 4.65 million ha with total production of 3.02 million tons and productivity being 7.00 q/ha (FAO, 2013) which is quite low because of its growing environments under rain fed water-limiting conditions and resource poor marginal lands along with several abiotic and biotic stresses affecting during different phases of crop growth. The major abiotic stresses affecting its production are moisture stress (sufficiency or deficiency) and temperature (high or low), salinity/alkalinity and acidity (Araujo *et al.,* 2015). In north India, pigeon pea experiences low temperature stress during winter months (December–January). The stress adversely affects growth, survival and reproductive capacity of plants if the minimum temperature falls below 5°C. At freezing temperature, intracellular water gets converted into ice, which in turn causes shrinkage of cells inside the plant, resulting in wilting and death of plants (Choudhary *et al.*, 2011). According to Wery *et al.* (1993), the intracellular ice in the plants causes cell dehydration and cell membrane destruction due to freeze-thaw cycle leading to death of the plants under cold conditions. Choudhary (2007) recorded data on buds/plant and flowers/plant in two-temperature environments under field condition (mean temperature: 16.4°C and 11.4°C). Low temperature stress (11.4°C) appeared to reduce the number of buds and flowers in each genotype. 'IPA 7-2' (a selection from a local land race 'Kudrat-3') was identified as the most tolerant on the basis of least reduction and better mean performance for the number of buds and flowers under low temperature condition. Preliminary investigation carried out at IIPR Kanpur also showed genotypic differences in the cold tolerance in pigeon pea (NICRA, 2016). Information's regarding cold stress and its impact in pigeon pea crop is very limited hence screening of a large number of pigeon pea genotypes for low temperature tolerance under

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controlled temperature condition is still needed to confirm and generate precise genetic information. It is also imperative to identify and validate morphological characters, physio-biochemical processes and indices using high-throughput controlled conditions and/or natural stress conditions for use as selection criteria in conventional and/or molecular breeding. Keeping the above facts in to consideration, the present experiment was formulated with the objectives to screen suitable genotypes of pigeon pea tolerant to cold stress based on morphological traits and ability to set flower and pods and yield performance.

Materials and Methods

Field experiments with 98 genotypes of pigeon pea were conducted in augmented design including checks (IPA203, Bahar,NDA-1,ICPL 7035, NDA-2, MAL-13, Pusa-9, Amar, MA-6, Azad, T-7, DA-11, MA-3, IPA 8F, IPA 9F, IPA- 9F vege., IPA 15F, IPA 16 F, KPL-43, KPL-44, IPA 2012-1, Allahabad local, Kudrat-3, Errama Chacha, Dholi-D, Rajendra Arhar, ICP-, 2883 B, ICP-12195, ICP 15-9-1, ICP 1637, VKG27/161, H-26, ICP-1997, ICP-12290, ICP2275, ICP 31076, JBT46/27, JBT 38/72, PH-1057, LRG 30, PBT/SSL 2/73, KPL-30, JBT 38/198, KPL 38, KPL 1034-31, IPA15-15, PDA-10, PBT/SSC/2/32, KPL 34, ICP 2073, IPAWD 10-1, IPACT-1, IPACT-2, IPACT-3, IPACT-4, IAF-114-2-1, IPAC 74-3, IPACT-5, IPAF114-2-2, IPACT-6, IPAHT-42, IPAB 10-66, IPAD-9, IPACT-7, IPACT-8, IPACT-9, IPAD 1-15, IPACT-10, IPACT-11, IPAHT-26, IPAHT-33, IPAD-10, IPACT-12, IPACT-13, IPACT-14, IPACT-15, IPAD2-8, IPACT-16, IPAD-8, IPACT-17, IPAHT-34, IPACT-24, IPACT-18, IPAHT-43, IPACT-19, IPAD 1-17, IPAHT-45, IPAD-6, IPAC-68, IPACT-21, IPA 17 B-11, IPAHT4-7, IPAC 79, IPA 17 B-12, IPACT-22, PAD-4, IPACT-23 and AGT-2) during *kharif* season 2018-19 and 2019-20 keeping line length of 4.00 meter and spacing of 60×20 cm with all recommended cultural practices at New Research Farm, ICAR-IIPR, Kanpur. Morphological, biochemical and yield parameters were recorded periodically. Morphological parameters including plant canopy structure with branching pattern with stature, scoring of cold injury at the apical meristems in exposed plants (3 each for every genotype), Number of flower drop and pod set during cold period in tagged plants with bagging (3 plants each), Post-regenerative capacity of meristems in exposed plants, Survival%, were recorded. In crop efficiency parameters *viz*: Nitrogen balance index (NBI), chlorophyll, flavonols and Anthocyanin's were measured using Dualex-Force A Leaf Clip Sensor (France). NDVI (Normalized Difference Vegetation Index) of all the genotypes was measured using Green Seeker (Trimble Agriculture) from 30 cm. distance in the day time in clear sky. Meteorological observations during cold stress like minimum and maximum temperature, relative humidity% and rainfall and cloudy weather were recorded from December to February during 2018-19 and 2019-20. The minimum temperatures below 10°C were recorded in total 33days and 32 days during December, January and February 2018-19 and 2019-20 respectively.

Parameters	Dec. 2018	Jan. 2019	Feb. 2019
Av. minimum temp.	12.60	9.51	10.02
RH%	93.78	73.97	66.53
Rain fall (m.m.)	0.00	1.80	10.40
No. of days with >10°C	09	16	08
Parameters	Dec. 2019	Jan. 2020	Feb. 2020
Av.minimum temp.	10.11	10.91	11.63
RH%	79.07	96.09	78.79
Rain fall (m.m.)	20	0.70	30.40
No. of days with <10°C	16.00	8.00	8.00

Yield attributes including pod number/plant, 100 seed weight and grain yield/plant were recorded at maturity after harvesting of crop. The data was analysed statistically using OPSTAT.

Results and Discussion

The effect of pigeon pea genotypes under cold stress was examined in terms of flower/pod drop and flower/pod retention, cold injury at the apical meristems and post regeneration of apical portion· the genotypes like IPA 15F, Dholi-D, JBT46/27, IPACT-6, IPACT-14, IPAC-1-17, IPACT-68, IPACT-22 showed >90% flower/pod drop and retained only 0-10% flowers/pods, >90% apical damage and<10% recovery of apical portion and were considered highly sensitive to cold stress. Genotypes namely MA-3, IPA 9F, ICP 15-9-1, VKG 27/161, H-26, ICPL 7035, LRG-30, PBT/SSL 2/73, KPL 1034-31, IPA 15-15, KPL 34, ICP 2073, IPACT-5, IPAB 10-66, IPACT-10, IPACT-11, IPAHT-26, IPACT-15, IPAD 2-8, IPACT-16, IPAD-8, IPACT-17, IPAHT-43, IPACT-21 showed >80% flower/pod drop and retained 10-20% flower/pods, >80% apical damage and <20% recovery of apical portion and considered moderately susceptible to cold stress (Table 1). Genotypes like NDA-2, MAL-13, ICP-2275, IPACT-2 showed around 20% flower/pod drop and have retained 60-80% flowers/pods, <20% apical damage and>80% recovery of apical portion were considered as the highly tolerant to cold stress. Genotypes including AMAR, MA-6, KPL-44, Rajendra Arhar, ICP 1997, ICP 12290, KPL 30, PBT/SSL 2/32, IPAWD 10-1, IPACT-3, IPAC 74-3, IPAF 114-2-2, IPACT-24, IPACT-19 showed around 50% flower/pod drop and have retained 40-60% flowers/pods, <50% apical damage and >50% recovery of apical portion and considered as the moderately tolerant to cold stress. Other genotypes including IPA 203, BAHAR, NDA-1, PUSA-9, AZAD, T-7, DA-11, IPA 8F, IPA-9F(Vege), IPA 16F, KPL-43, IPA 2012-1, Allahabad Local, Kudrat-3, Errama Chacha, ICP 2883B, ICP 12195, ICP 1637, ICP 31076, JBT 38/72, PH 1057, JBT 38/198, KPL 38, PDA-10, IPACT-4, IPAB 10-66, IPA 9, IPACT-7, IPACT-8, IPACT-9, IPAD 1-15, IPAH-33, IPAD-10, IPAH-34, IPACT-18, IPAD-6, IPAHT-4-7, IPAC-79, IPACT-23, AGT-2 have shown mild reaction with 60-80% flower/pod drop and 20-40% flowers/pods retention, >60% apical damage and <40% recovery of apical portion) to cold stress (Table 1). Our results showed

very high genotypic variation against the cold stress in respect of flower/pod drop and their retention and very much similar with the findings of Singh *et al.* (1997) who reported the bud and flower drop during severe cold at Faizabad (U.P.) condition. The cold susceptible genotypes like IPA 15F, Dhule-D, JBT46/27, IPACT-6, IPACT-14, IPAC-1-17, IPACT-68, IPACT-22 showed the 90% plant mortality and plant survival is only 10% and showed enormous variation in pigeon pea crop against the cold stress and similar results were also recorded in a field study in china (Yong *et al.,* 2002). The cold stress adversely affects growth, survival and reproductive capacity of plants if the minimum temperature falls below 5°C, a freezing temperature converted, intracellular water into ice, which in turn causes shrinkage of cells inside the plant, resulting in wilting and death of plants. According to Wery *et al*. (1993), the intracellular ice in the plants causes cell dehydration and cell membrane destruction due to freeze-thaw cycle leading to death of the plants under cold conditions. The tolerant genotypes (highly/ moderate) of pigeon pea including NDA-2, MAL-13, ICP2275, IPACT-2, AMAR, MA-6, KPL-44, Rajendra Arhar, ICP 1997, ICP 12290, KPL 30, PBT/SSL 2/32, IPAWD 10-1, IPACT-3, IPAC 74-3, IPAF 114-2-2, IPACT-24, IPACT-19 showed the presence of genetic variability vis-à-vis cold tolerance and our results are supported with the findings of Sandhu *et al*. (2007) who screened for cold tolerance in a set of 480 pigeon pea lines at PAU, Ludhiana and found 32 genotypes cold tolerant as the plants retained their normal morphology with intact floral buds. Singh *et al*. (1997) observed that long-duration cultivars are well-adapted to cold situations because of their inherent genetic mechanism to cope up with very low temperature during reproductive stages. Choudhary (2007) noted that low temperature stress (11.4°C) appeared to reduce the number of buds and flowers in each genotype. 'IPA 7-2' (a selection from a local land race 'Kudrat-3') was identified as the most tolerant on the basis of least reduction and better mean performance for the number of buds and flowers under low temperature condition. However, the other genotype 'Bahar' also appeared at par with the 'IPA 7-2'. The research conducted at the IIPR, Kanpur (Annual Report, 2008-09) revealed that low temperature primarily affects development and growth of flower buds. In some sensitive genotypes such as 'IPA 209' and 'IPA 06-1', filaments of stamens fail to enlarge at low temperature and thus affect opening of flowers. Pollen dehiscence does not occur too, although pollens are fully

Table 1: Average flower/pod drop and retention %, apical damage and recovery% in pigeon pea genotypes after exposer of cold stress.

Pigeon pea genotypes	Flowers/pods drop after exposer of cold stress (%)	Retention of flowers /pods after exposer of cold stress (%)	Apical damage %	Regeneration/ recovery %	Tolerance/ sensitivity index
IPA 15F, Dhule-D, JBT46/27, IPACT-6, IPACT-14, IPAC-1-17, IPACT-68, IPACT-22	>90	0-10	>90	> 10	Highly sensitive to cold
MA-3, IPA 9F, ICP 15-9-1, VKG 27/ 161, H-26,ICPL 7035, LRG-30, PBT/SSL 2/73, KPL 1034-31, IPA 15-15, KPL 34, ICP 2073, IPACT-5, IPAB 10-66, IPACT-10, IPACT-11, IPAHT-26, IPACT-15, IPAD 2-8, IPACT-16, IPAD-8, IPACT-17, IPAHT-43, IPACT-21	>80	10-20	>80	>20	Moderately susceptible to cold
IPA 203, BAHAR, NDA-1, PUSA-9, AZAD, T-7, DA-11, IPA 8F, IPA(Vege), IPA 16F, KPL -43, IPA 2012-1, Allahabad Local, Kudrat-3, Errama Chacha, ICP 2883B, ICP 12195, ICP 1637, ICP 31076, JBT 38/72, PH 1057, JBT 38/198, KPL 38, PDA-10, IPACT-4, IPAB 10-66, IPA 9, IPACT-7, IPACT-8, IPACT-9, IPAD 1-15, IPAH-33, IPAD-10, IPAH-34, IPACT-18, IPAD-6, IPAHT-4-7, IPAC-79, IPACT-23, AGT-2	60-80	20-40	>60	>40	Mild reaction to cold
Amar, MA-6,KPL-44, Rajendra Arhar, ICP 1997, ICP 12290, KPL 30, PBT/SSL 2/32, IPAWD 10-1, IPACT-3, IPAC 74-3, IPAF 114-2-2, IPACT-24, IPACT-19	50	40-60	<50	>50	Moderately tolerant to cold
NDA-2, MAL-13, ICP2275, IPACT-2	20	60-80	< 20	> 80	Tolerant to cold

C.V. -20.70; C.D. for control Treatment-8.95; C.D. for Test Treat.in same block-13.70; C.D. for Test Treat. Not in same block-16.50; C.D. for Test Treat. And a control- 09.52.

fertile. As a consequence, unfertilized flowers wither and fall down, resulting in no pod formation in these genotypes under low temperature.

Plant efficiency parameters including nitrogen balance index (NBI), chlorophyll, flavonols, anthocyanin's and normalized difference vegetation index (NDVI) showed varying trends with cold susceptible and cold tolerant group of pigeon pea genotypes (Table 2). The nitrogen balance index, chlorophyll and flavonols showed higher values in tolerant group whereas lower in susceptible group indicating there by the better uptake of nitrogen, more chlorophyll for better photosynthetic efficiency and more flavonols for better resistance against the cold stress in tolerant genotypes over susceptible group of genotypes. Anthocyanin's are deposited in leaves against the cold stress (Kumar *et al.,* 2010) and in our studies too the cold susceptible genotypes showed relatively higher anthocyanin's over tolerant group of genotypes (Table 2). Normalized difference vegetation index is the measure of density of greenness over the land area of the particular vegetation and in our results it is significantly higher in tolerant genotypes over susceptible genotypes. Our results are supported with the findings of Hajihashemi *et al.* (2018) in S*tevia rebaudiana* and Bhat *et al.* (2022) in legumes crops who have reported the reduction in net photosynthesis, intercellular CO_2, water use efficiency, chlorophyll a andb and carotenoid contents due to cold stress. Alterations in the expression of S-Adenosy lmet hionine synthetase gene, polyamines and antioxidant activity are also reported in pigeon pea crop when imposed to cold stress (Radadiya *et al.,* 2016).

Yield attributes including pod number/plant,100 grain weight and yield/plant showed wide range of genotypic variation in pigeon pea genotypes evaluated irrespective of the susceptible/tolerant tendency against cold stress. The pod number/plant ranges from 90 to 610, 100 grain weight ranges from 7.27 to 28.80 g and yield/plant ranges from 23 to 239.50 g among the pigeon pea genotypes evaluated and showed no definite trend with varying degree of tolerance/susceptibility of genotypes against cold stress. The pigeon pea genotypes tested under cold stress when

Table 2: Plant efficiency attributes in pigeon pea genotypes under cold stress.

Pigeon pea genotypes	Tolerance/ susceptibility index	NBI	Chlorophyll	Flavonols	Anthocyanin	NDVI
IPA 15F, Dholi-D, JBT46/27, IPACT-6, IPACT-14, IPAC-1-17, IPAC-68, IPACT-22	Highly sensitive to cold	23.87	39.70	1.605	0.0041	0.40
MA-3, IPA 9F, ICP 15-9-1, VKG 27/161, H-26,ICPL 7035, LRG-30, PBT/SSL 2/73, KPL 1034-31, IPA 15-15, KPL 34,ICP 2073, IPACT-5, IPAB 10-66, IPACT-10, IPACT-11, IPAHT-26, IPACT-15, IPAD 2-8, IPACT-16, IPAD-8, IPACT-17, IPAHT-43, IPACT-21	Moderately susceptible to cold	25.01	41.60	1.65	0.0033	0.50
IPA 203, BAHAR, PUSA-9, AZAD, T-7, DA-11, IPA 8F, IPA(Vege), IPA 16F, KPL-43, IPA 2012-1, Allahabad Local, Kudrat-3, Errama Chacha, ICP 2883B, ICP 12195, ICP 1637, ICP 31076, JBT 38/72, PH 1057, JBT 38/198, KPL 38, PDA-10, IPACT-4, IPAB 10-66, IPA 9, IPACT-7, IPACT-8, IPACT-9, IPAD 1-15, IPAH-33, IPAD-10, IPAH-34, IPACT-18, IPAD-6, IPAHT-4-7, IPAC-79, IPACT-23, AGT-2, IPACT-1	Mild reaction to cold	25.15	41.64	1.66	0.003	0.50
AMAR, NDA-1, MA-6, KPL-44, Rajendra Arhar, ICP 1997, ICP 12290, KPL 30, PBT/SSL 2/32, IPAWD 10-1, IPACT-3, IPAC 74-6, IPAF 114-2-2, IPACT-24, IPACT-19	Moderately tolerant to cold	25.24	41.87	1.68	0.003	0.50
NDA-2, MAL-13, ICP2275, IPACT-2	Highly tolerant to cold	25.83	42.00	1.72	0.00	0.67
C.V.		6.14	9.076	4.10	-	34.0
C.D. for control Treat		2.78	4.06	0.075		0.016
-C.D. for Test Treat in same block-		7.36	10.74	0.198		0.043
C.D. for Test Treat. Not in same block-		8.23	12.01	0.221		0.048
C.D. for Test Treat. And a control Treat-		6.06	8.85	0.163		0.036

Table 3: Yield performance of contrasting pigeon pea genotypes under cold stress.

Cold tolerant genotypes	yield/plant (g)	Cold susceptible genotypes	yield/plant (g)
NDA-2	156.60	IPA 15 F	174.00
MAL-13	42.50	Dholi-D	16.50
ICP-2275	239.50	JBT46/27	83.75
IPACT-2	57.20	IPACT-6	57.50
MA-6	88.00	IPACT-14	51.70
NDA-1	126.00	IPAC-1-17	31.65
IPACT-3	37.20	IPAC-68	36.65
IPACT-24	88.50	IPACT-22	74.20

classified on the basis of their flower/pod retention efficiency, the eight (08) tolerant genotypes are NDA-2, MAL-13, ICP-2275, IPACT-2, MA-6, NDA-1, IPACT-3 and IPACT-24 and eight (08) susceptible genotypes are IPA 15 F, Dholi-D, JBT46/27, IPACT-6, IPACT-14, IPAC-1-17, IPAC-68 and IPACT-22. The yield performance of these contrasting genotypes are as in (Table 3). The average yield/plant of pigeon pea genotypes tolerant to cold stress is considerably higher than the susceptible group of pigeon pea genotypes indicating there by the better efficacy of tolerant group to maintain normal vegetative and reproductive capacity of the plants over susceptible group. Rana *et al.* (2016) reported that in pigeon pea crop at very low temperature, the photo synthetic efficiency is retarded owing to chilling temperature, moisture stress, internal injury and production of reactive oxygen species (ROS) and the reproductive capacity of most of the genotypes is also reduced if the temperature falls below 10°C (Sultana *et al.,* 2014) leading to the impairment of growth and development of flower buds, the enlargement of filaments and the process of anthesis (Choudhary, 2007). Low temperature also precludes pollen dehiscence, resulting in failure of pod formation in sensitive genotypes. The initiation and development of floral buds, number of blossomed flowers and pod setting at low temperature (Singh and Singh, 2010) may be used as traits to discriminate between sensitive and tolerant genotypes of pigeon pea. These are the possible reasons for flower/pod drop and its retention in pigeon pea genotypes and determines the tolerance/susceptibility against the cold stress. Screening of a large number of pigeon pea genotypes for low temperature tolerance under controlled temperature condition is still needed to confirm these findings and generate more information's.

Conclusion

Pigeon pea genotypes reaction against the exposer of cold stress in terms of flower/pod drop and flower/pod retention, cold injury at the apical meristems and post regeneration of apical portion was as under, the genotypes like IPA 15F, Dhule-D, JBT46/27, IPACT-6, IPACT-14, IPAC-1-17, IPACT-68, IPACT-22 showed >90% flower/pod drop and retained only 0-10% flowers/pods, >90% apical damage and<10% recovery of apical portion and were considered highly sensitive to cold stress. Genotypes namely MA-3, IPA 9F, ICP 15-9-1, VKG 27/161, H-26, ICPL 7035, LRG-30, PBT/SSL 2/73, KPL 1034-31, IPA 15-15, KPL 34, ICP 2073, IPACT-5, IPAB 10-66, IPACT-10, IPACT-11, IPAHT-26, IPACT-15, IPAD 2-8, IPACT-16, IPAD-8, IPACT-17, IPAHT-43, IPACT-21 showed >80% flower/pod drop and retained 10-20 % flower/pods,>80%apical damage and <20% recovery of apical portion and considered moderately susceptible to cold stress. Genotypes NDA-2, MAL-13, ICP-2275, IPACT-2 showed around 20-40% flower/pod drop and have retained 60-80% flowers/pods, <20% apical damage and >80% recovery of apical portion were considered as the highly tolerant to cold stress. The nitrogen balance index, chlorophyll and flavonols showed higher values in the tolerant group whereas lower in the susceptible group. The average yield/plant of pigeon pea genotypes tolerant to cold stress is considerably higher than the susceptible group of pigeon pea genotypes.

Conflict of interest

All authors declared that there is no conflict of interest.

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