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Physiological Characterization of Cold Tolerant and Susceptible Pigeon Pea [*Cajanus cajan* (L.) Millsp.] Genotypes

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ABSTRACT

Background: The cold stress adversely affects the growth, survival and reproductive capacity of plants if the minimum temperature falls below 5°C. Information's regarding cold stress and its impact on morphological characters, physio-biochemical constituents and yield attributes in cold tolerant and susceptible pigeon pea genotypes are very limited. Keeping the above facts in to consideration, the experiments were conducted.

Methods*:* Field experiments were conducted during 2018-19 and 2019-20 with 34 genotypes of pigeon pea including checks (NDA-1, Bahar, IPA203, NDA-2, MAL-13, ICP2275, IPACT-2, MA-6, IPACT-3, IPACT-24, Rajendra Arhar, IPA15F, Dholi-D, JBT46/27, IPACT-6, IPACT-14, IPAD1-17, IPAC 68, IPACT 22, ICPL 7035 IPA19-101, IPA19-102, IPA19-103, IPA19-104, IPA19-105, IPA19-106, IPA19-107, IPA19-108, IPA19-109, IPA19-110, IPA19-111, IPA19-112, IPA19-113, IPA19-114) in RBD at ICAR-IIPR, Kanpur. Morphological and yield Characters including plant height, scoring of cold injury in exposed plants and Number of flower drop and pod set during the cold period in tagged plants were recorded. In biochemical/ crop efficiency parameters *viz:* Nitrogen balance index, chlorophyll, flavonols, anthocyanin's and NDVI were measured. The photosynthetic rate, stomatal conductance, and transpiration rate were measured at the pod formation stage. Anti-oxidant enzymes activity was assayed as per standard methods. Meteorological observations during Cold stress were recorded.

Result: Based on flower/pod drop and retention the genotypes like IPA 15F, Dholi dwarf, JBT46/27, IPACT-6, IPACT-14, IPAC-1-17, IPACT-68, IPACT-22, IPA 19-101, IPA 19-102, IPA 19-103, IPA 19-104, IPA 19-105, IPA 19-107, IPA 19-108, IPA 19-109, IPA 19-110, IPA 19-111 and IPA 19-113 were found susceptible to cold stress and retaining an average 51.67 % flowers/pods. Genotypes like NDA-2, MAL-13, ICP2275, IPACT-2, MA-6, IPACT-3, IPACT-24, Rajendra arhar, IPA19-106, IPA19-112 and IPA19-114 have retained more than an average of 67.48% flowers/pods are considered tolerant to cold stress. Amongst the tolerant group NDA-2, MAL-13, ICP2275, IPACT-2, IPA19-106, IPA19-112 retained the flower/pod more than the average of check genotypes (67.57%). Biochemical/ plant efficiency parameters were superior in tolerant group over susceptible group.

Key words: Anti-oxidants, Cold stress, Cold susceptible, Cold tolerance, Flower/pod drop, Flower/pod retention, Genotypes, Photosynthetic efficiency.

INTRODUCTION

In India, the area occupied by pigeonpea is about 4.78 million ha with a total production of 3.43 million tons and productivity being 850 kg/ha (https://agricoop.nic.in/2023) 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, pigeonpea experiences low-temperature stress during winter months (December-January). The stress adversely affects the growth, survival and reproductive capacity of plants if the minimum temperature falls below 5°C. At the 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 plants causes cell dehydration and cell membrane destruction due to the freeze-thaw cycle leading to the death of the plants under cold conditions. Choudhary (2007) recorded data on buds/plants and flowers/plants in two-temperature environments under field conditions (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 landrace 'Kudrat-3') was identified as the most tolerant based on the least reduction and better mean performance for the number of buds and flowers under low-temperature conditions. The preliminary investigation carried out at IIPR

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Kanpur also showed genotypic differences in the cold tolerance of pigeonpea (NICRA, 2016). Information regarding cold stress and its impact on pigeonpea crop is limited, Tiwari *et al*. (2022) found that the genotypes like IPA 15F, Dholi Dwarf, JBP46/27, 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 like NDA-2, MAL-13, ICP-2275 and 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. Keeping the above facts into consideration, the present experiment was formulated with the objectives to study the cold tolerant and cold susceptible pigeonpea genotypes for their morpho-physiological characterization and ability to set flowers and pods under field conditions.

MATERIALS AND METHODS

Field experiments were conducted during 2018-19 and 2019-20 with 34 genotypes of pigeon pea including checks (NDA-1, Bahar, IPA203, NDA-2, MAL-13, ICP2275, IPACT-2, MA-6, IPACT-3, IPACT-24, Rajendra Arhar, IPA15F, Dholi-D, JBT46/27, IPACT-6, IPACT-14, IPAD1-17, IPAC 68, IPACT 22, ICPL 7035, IPA19-101, IPA19-102, IPA19-103, IPA19-104, IPA19-105, IPA19-106, IPA19-107, IPA19-108, IPA19-109, IPA19-110, IPA19-111, IPA19-112, IPA19-113, IPA19-114) in Randomized Block Design keeping plot size of 4.00 × 3.00 meter and spacing of 60×20 cm. The sowing of experimental genotypes was completed in the first fortnight of July in both the year with all recommended agronomic practices and fertilizer doses at the New Research Farm, ICAR-IIPR, Kanpur. Morphological and yield characters including plant height, scoring of cold injury at the apical meristems in exposed plants (3 each for every genotype) and number of flower drop and pod set during the cold period in tagged plants with bagging (3 plants each), were recorded (Fig 1).

In biochemical/ 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 daytime in the clear sky. The photosynthetic rate, stomatal conductance and transpiration rate were measured at the pod formation stage using IRGA-Portable Photosynthesis Measurement system LICOR-6400 (USA) between 11.0AM to 2.00 PM.

Anti-oxidant enzyme peroxidase activity was assayed as an increase in optical density due to the oxidation of guaiacol to tetra guaiacol (Castillo *et al.*,1984), Super oxide dismutase (SOD) assay was based on the formation of blue colored formazone by nitro blue tetrazolium and O^-_2 radical which absorbs at 560 nm and the enzyme (SOD) decreases this absorbance due to reduction in the formation of O^-_2 radical by the enzyme (Dhindsa *et al.*,1981). Meteorological observations during Cold stress like Minimum and maximum temperature, relative humidity % and rainfall were recorded from November,15 to February,15 in both the year and given below in tabular form:

Parameters	Dec.2018	Jan.2019	Feb.2019
Av.minimum temp.	12.60	9.51	10.02
RH%	93.78	73.97	66.53
Rainfall (mm)	0.00	1.80	10.40
No. of days with >10°	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
Rainfall (mm)	20	0.70	30.40
No. of days with >10°C	16.00	8.00	8.00

Fig 1: Tolerant (NDA-2 and ICP-2275) and susceptible (Dholi dwarf and ICPL-7035) genotypes against cold stress.

The yield attributes and yield was recorded at maturity/ harvesting. Two-year data were pooled and statistically analysed using OPSTAT software.

RESULTS AND DISCUSSION

The Reaction of pigeon pea genotypes against exposure to cold stress in terms of flower/pod drop and/or flower/ pod retention is given in Table 1. Based on flower/pod drop and retention the genotypes like IPA 15F, Dholi dwarf, JBT46/27, IPACT-6, IPACT-14, IPAC-1-17, IPACT-68, IPACT-22, IPA 19-101, IPA 19-102, IPA 19-103, IPA 19-104, IPA 19-105, IPA 19-107, IPA 19-108, IPA 19-109, IPA 19-110, IPA 19-111 and IPA 19-113 were found susceptible to cold stress and retaining an average 51.67 % flowers/pods. Amongst susceptible genotypes Dholi- D, JBT 46/27, IPAD1-17, IPACT-22, IPA19-102 and IPA19-103 recorded very low

Table 1: Pod retention per cent after exposure to cold in pigeon pea genotypes.

Genotypes	No. of flowers tagged/ plant (Average)	Average nos. of pods retained/plant (Average)	% age of retention of pods/plant (Average)
		Tolerant	
NDA-1 (Check)	63.73	43.26	67.88
Bahar (Check)	57.33	37.30	65.06
IPA203 (Check)	65.00	45.35	69.77
NDA-2	65.0	45.41	69.86
MAL-13	65.66	46.17	70.31
ICP2275	63.00	43.58	69.14
IPACT-2	63.66	43.70	68.40
MA-6	57.00	37.51	65.80
IPACT-3	63.00	42.50	67.46
IPACT-24	53.33	32.25	60.47
Rajendra arhar	65.33	43.83	67.09
IPA 19-106	65.33	44.46	68.05
IPA 19-112	66.00	45.00	68.18
IPA 19-114	61.00	41.00	67.21
Mean	62.45	42.24	67.48
		Susceptible	
ICPL 7035 (Check)	68.0	37.25	54.77
IPA 15F	69.33	40.66	58.64
Dholi-D	43.33	12.75	29.42
JBT 46/27	65.66	34.67	52.80
IPACT-6	69.33	38.25	55.17
IPACT-14	67.33	38.25	56.80
IPAD1-17	69.66	36.17	51.92
IPAC 68	61.33	37.46	61.04
IPACT- 22	65.00	29.86	45.93
IPA 19-101	65.66	35.16	53.54
IPA 19-102	59.00	26.00	44.06
IPA 19-103	59.30	28.75	48.48
IPA 19-104	74.66	43.75	58.59
IPA 19-105	62.33	30.78	49.38
IPA 19-107	61.00	29.41	48.21
IPA 19-108	61.67	30.60	49.61
IPA 19-109	65.66	34.66	52.78
IPA 19-110	65.66	38.16	58.11
IPA 19-111	67.33	35.50	52.72
IPA 19-113	64.00	32.91	51.42
Mean	64.26	33.55	51.67
C.D.	-	8.332	14.652
SE(m)		2.391	5.177
SE(d)		3.951	7.322
C.V.		18.177	24.183

retention of flower/ pod as compared to check ICPL-7035 (Table 1).

Genotypes like NDA-2, MAL-13, ICP2275, IPACT-2, MA-6, IPACT-3, IPACT-24, Rajendra arhar, IPA19-106, IPA19-112 and IPA19-114 have retained more than an average of 67.48% flowers/pods are considered the tolerant to cold stress. Amongst the tolerant group NDA-2, MAL-13, ICP2275, IPACT-2, IPA19-106, IPA19-112 retained the flower/pod more than the average of check genotypes (67.57%) including NDA-1(Check), Bahar (Check) and IPA203 (Check). Conclusively, genotypes NDA-2, MAL-13, ICP2275, IPACT-2, IPA19-106, IPA19-112 are highly tolerant to cold stress and genotypes Dholi- D, JBT 46/27, IPAD1-17, IPACT-22, IPA19-102 and IPA19-103 are highly susceptible to cold stress. Our results showed very high genotypic variation against the cold stress in respect of flower/pod drop and their retention and very much similar to the findings of Singh *et al*. (1997) who reported the bud

Table 2: Morphological characters as influenced by cold stress.

Genotypes	Plant height (in cm)	NBI	Chlorophyll	Flavonols	Anthocyanins
			Tolerant		
NDA-1	178	24.8	42.3	1.71	0.0
Bahar	260	25.2	46.8	1.76	0.0
IPA203	203	31.7	45.3	1.73	0.0
NDA-2	213	25.6	44.5	1.74	0.0
MAL-13	197	29.8	38.0	1.47	0.0
ICP2275	206	23.3	39.1	1.68	0.0
IPACT-2	138	23.1	41.4	1.80	0.01
MA-6	182	22.8	38.1	1.74	0.0
IPACT-3	160	20.9	37.5	1.82	0.01
IPACT-24	173	21.6	38.4	1.78	0.0
Rajendra arhar	193	20.5	39.8	1.84	0.0
IPA 19-106	208	28.7	42.6	1.48	0.0
IPA 19-112	214	25.9	46.2	1.78	0.0
IPA 19-114	199	19.8	37.9	1.82	0.0
Mean	194.57	24.55	41.28	1.72	0.001
			Susceptible		
ICPL 7035	190	24.5	40.1	1.42	0.0
IPA 15F	193	21.7	38.2	1.76	0.0
Dholi-D	92	17.9	30.7	1.71	0.0
JBT 46/27	198	24.7	44.3	1.79	0.0
IPACT-6	173	26.5	42.8	1.50	0.0
IPACT-14	191	27.4	44.5	1.62	0.0
IPAD1-17	176	18.8	33.7	1.79	0.0
IPAC 68	187	25.5	46.4	1.82	0.0
IPACT- 22	197	24.2	42.4	1.75	0.0
IPA 19-101	207	26.2	42.7	1.63	0.0
IPA 19-102	192	24.6	41.9	1.70	0.0
IPA 19-103	187	22.1	36.9	1.67	0.0
IPA 19-104	197	26.9	62.2	1.60	0.0
IPA 19-105	216	25.2	39.8	1.58	0.0
IPA 19-107	207	26.1	44.3	1.70	0.0
IPA 19-108	208	26.0	43.7	1.68	0.0
IPA 19-109	206	25.6	43.5	1.70	0.0
IPA 19-110	267	20.1	35.8	1.78	0.0
IPA 19-111	203	25.9	44.1	1.70	0.0
IPA 19-113	202	21.7	13.2	1.62	0.0
Mean	194.45	24.08	40.56	1.67	0.00
C.D.	3.148	0.972	0.967	0.031	-
SE(m)	1.112	0.344	0.342	0.011	
SE(d)	1.573	0.486	0.483	0.016	
C.V.	0.988	2.429	1.449	1.114	

and flower drops during severe cold at Faizabad(U.P.) condition. The cold susceptible genotypes like IPA 15F, Dhole-D, JBT46/27, IPACT-6, IPACT-14, IPAC-1-17, IPACT-68, IPACT-22, IPA 19-101, IPA 19-102, IPA 19-103, IPA 19-104, IPA 19-105, IPA 19-107, IPA 19-108, IPA 19-109, IPA 19-110, IPA 19-111 and IPA 19-113 showed about the 48.33% plant mortality and plant survival is only 51.67% 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 the 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 plants causes cell

Table 3: Effect of cold stress on photosynthetic efficiency and NDVI.

Genotypes	NDVI	Photo synthetic rate (µmol $co_2m^{-2}s^{-1}$)	Stomatal conductance (mmol $m^{-2}s^{-1}$)	Transpiration rate (mmol $m^{-2}s^{-1}$)
		Tolerant		
NDA-1	0.79	8.110	1.261	21.439
Bahar	0.79	4.116	0.847	20.440
IPA203	0.80	7.434	1.376	19.397
NDA-2	0.75	6.037	0.962	20.925
MAL-13	0.80	4.795	1.225	20.629
ICP2275	0.75	9.360	1.294	20.925
IPACT-2	0.71	8.030	1.208	19.839
MA-6	0.70	5.561	0.294	20.684
IPACT-3	0.75	4.351	0.134	20.957
IPACT-24	0.61	9.229	0.131	20.927
Rajendra arhar	0.58	12.95	0.302	20.167
IPA 19-106	0.73	5.591	0.111	20.665
IPA 19-112	0.69	14.56	0.254	21.187
IPA 19-114	0.72	13.209	0.257	20.897
Mean	0.73	8.095	0.69	20.65
		Susceptible		
ICPL 7035	0.72	7.563	0.425	20.592
IPA 15F	0.54	9.357	0.372	20.512
Dholi-D	0.52	5.279	0.062	20.216
JBT 46/27	0.68	4.952	0.280	20.505
IPACT-6	0.70	2.923	0.049	20.441
IPACT-14	0.73	8.089	0.372	20.456
IPAD1-17	0.64	7.244	0.168	19.448
IPAC 68	0.55	6.410	0.052	20.665
IPACT- 22	0.70	6.314	0.165	19.797
IPA 19-101	0.72	6.005	0.271	20.710
IPA 19-102	0.74	3.074	0.259	20.674
IPA 19-103	0.74	4.51	0.226	20.584
IPA 19-104	0.76	4.736	0.301	19.620
IPA 19-105	0.70	6.83	0.06	20.660
IPA 19-107	0.73	5.621	0.122	20.586
IPA 19-108	0.74	5.741	0.221	19.661
IPA 19-109	0.72	6.61	0.96	20.251
IPA 19-110	0.74	6.81	0.244	20.660
IPA 19-111	0.62	7.30	0.236	20.652
IPA 19-113	0.70	6.72	0.074	19.567
Mean	0.68	6.104	0.245	20.31
C.D.	0.042	0.558	0.064	0.222
SE(m)	0.015	0.197	0.023	0.078
SE(d)	0.021	0.279	0.032	0.111
C.V.	3.614	3.507	9.045	0.666

dehydration and cell membrane destruction due to the freeze-thaw cycle leading to the death of the plants under cold conditions. The tolerant genotypes 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 .

Photographs showing tolerant (NDA-2 and ICP-2275) and susceptible (Dholi dwarf and ICPL-7035) genotypes against cold stress.

With the findings of Sandhu *et al*. (2007) who screened for cold tolerance in a set of 480 pigeon pea lines at PAU, Ludhiana found that 32 genotypes were cold tolerant as the plants retained their normal morphology with intact floral buds. They suggested utilizing these genotypes to enhance the cold tolerance of sensitive varieties and study the

Table 4: Effect of cold stress on anti-oxidant enzyme activity and grain yield.

Genotypes	Peroxidase	Super oxide dismutase	100 seed wt.	Grain yield
			Tolerant	
NDA-1	4.61	1.30	11.23	1.353
Bahar	3.10	2.06	11.56	0.627
IPA203	0.53	1.00	15.82	0.545
NDA-2	0.01	3.06	15.55	0.678
MAL-13	0.86	1.71	13.05	0.532
ICP2275	0.01	1.05	09.80	0.647
IPACT-2	1.50	1.20	14.63	0.607
MA-6	0.09	1.62	16.77	0.580
IPACT-3	0.03	2.26	15.31	0.547
IPACT-24	0.04	1.90	12.28	0.495
Rajendra arhar	0.06	1.42	13.04	0.567
IPA 19-106	1.520	1.76	12.60	0.488
IPA 19-112	1.620	1.92	13.12	0.642
IPA 19-114	1.760	1.84	13.22	0.652
Mean	1.140	1.850	14.41	0.683
			Susceptible	
ICPL 7035	3.41	2.21	15.55	0.558
IPA 15F	0.01	1.64	11.91	0.775
Dholi-D	1.53	2.21	9.94	0.075
JBT 46/27	0.09	1.70	12.79	0.303
IPACT-6	2.08	2.30	11.35	0.542
IPACT-14	0.04	1.53	10.22	0.754
IPAD1-17	0.01	1.56	09.75	0.564
IPAC 68	0.09	1.76	17.13	0.309
IPACT- 22	1.36	1.20	11.75	0.496
IPA 19-101	0.052	1.30	10.54	0.864
IPA 19-102	0.051	2.18	12.17	0.452
IPA 19-103	0.040	1.20	10.95	0.784
IPA 19-104	1.240	1.44	9.97	0.846
IPA 19-105	1.860	2.10	13.10	0.751
IPA 19-107	0.640	1.50	12.68	0.484
IPA 19-108	0.430	1.64	9.91	0.883
IPA 19-109	0.524	1.76	10.63	0.775
IPA 19-110	0.072	1.54	10.60	0.754
IPA 19-111	0.084	1.34	10.94	0.795
IPA 19-113	0.084	1.44	11.97	0.791
Mean	0.682	1.677	11.69	0.627
C.D.	0.054	0.158	0.154	25.988
SE(m)	0.019	0.056	0.055	9.183
SE(d)	0.027	0.079	0.077	12.987
C.V.	3.712	4.084	0.756	2.238

genetics of cold tolerance. Singh *et al*. (1997) observed that long-duration cultivars are well-adapted to cold situations because of their inherent genetic mechanism to cope with low temperatures during reproductive stages. Choudhary (2007) recorded data on buds/plant and flowers/ plant in two-temperature environments under field conditions (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 landrace 'Kudrat-3') was identified as the most tolerant based on the least reduction and better mean performance for the number of buds and flowers under low-temperature conditions. 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 the 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 temperatures and thus affect the opening of flowers. Pollen dehiscence does not occur too, although pollens are fully fertile. As a consequence, unfertilized flowers wither and fall, resulting in no pod formation in these genotypes under low temperatures.

The effects of cold stress on biochemical/plant efficiency parameters including NBI, Chlorophyll, Flavonols, Anthocyanin and plant height were studied and no difference was observed in plant height. The Nitrogen Balance Index, chlorophyll, Flavonols and Anthocyanins were slightly higher in the tolerant group as compared to the susceptible group (Table 2). The Nitrogen balance index, chlorophyll and flavonols showed higher values for the tolerant group whereas lower in the 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. Anthocyanins are deposited in leaves against cold stress (Kumar *et al*., 2010) and in our studies, too the cold-tolerant genotypes showed relatively higher anthocyanins over the susceptible group of genotypes (Table 2) thereby indicating their indirect role in cold tolerance among the pigeonpea genotypes. Normalized difference vegetation index is the measure of the 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. Plant efficiency parameters like photosynthetic rate, stomatal conductance and transpiration rate, showed significantly higher values in the tolerant group over the susceptible group indicating the superiority of cold tolerant genotypes in photosynthetic efficiency over cold susceptible genotypes (Table 3). Our results are supported by the findings of Hajihashemi *et al.* (2018) who reported the reduction in efficiency of photosystems I and II, net photosynthesis, intercellular Co_2, water use efficiency, chlorophyll a and b and carotenoid contents in *Stevia rebaudiana* due to cold stress. These physiological traits might be considered as the parameter for cold tolerance (Table 3 and Fig 1). In tomatoes low-temperature stress negatively affects both the growth and development of plants through damage to photosynthetic components and the inhibition activity of antioxidant enzymes as reported by Hee Ju Lee (2021). Our results are also in close conformity with the findings of Fanhang *et al*. (2020) who reported a reduction in the photosynthetic efficiency of tung tree seedlings, affecting the formation of the internal structure of the plant leaves and destroying the integrity and function of chloroplast under long term low-temperature conditions. Cold/freezing temperatures were also reported to reduce the chlorophyll, fluorescence and photosynthetic parameters in *Camellia veiningensis* and *C. oleifera* seedlings (Hongyun Xu *et al*., 2022).

Anti-oxidant enzymes including peroxidase and Super Oxide Dismutase also showed higher activity in the tolerant group as compared to susceptible group (Table 4). Tolerant group of varieties showed higher 100 seed weight and grain yield per plant over susceptible group (Table 4). Enhancement in SOD and Guaiacol Peroxidase under cold stress which protects from Reactive Oxygen Species (ROS) act as free radical scavenger was reported in *Ocimum basilicum* L and also similar with our findings in pigeonpea (Ramin Rezaie *et al*., 2020). In *Brassica oleracea* crops the cold stress affects adversely the photosynthetic activity, antioxidant defense system and finally the yield of the crop (Pilar soengas *et al*., 2018). Our findings are also similar to the above findings in pigeon pea genotypes evaluated.

CONCLUSION

For physiological Characterization of the cold tolerant and susceptible pigeon pea [*Cajanus cajan* (L.) Millsp.] genotypes based on morpho-physiological traits, biochemical attributes and ability to set flower and pods and yield performance, field experiments were conducted during 2018-19 and 2019-20 with 34 genotypes of pigeon pea including checks (NDA-1, Bahar, IPA203, NDA-2, MAL-13, ICP2275, IPACT-2, MA-6, IPACT-3, IPACT-24, Rajendra Arhar, IPA15F, Dholi-D, JBT46/27, IPACT-6, IPACT-14, IPAD1-17, IPAC 68, IPACT 22, ICPL 7035 IPA19-101, IPA19-102, IPA19-103, IPA19-104, IPA19-105, IPA19-106, IPA19-107, IPA19-108, IPA19-109, IPA19-110, IPA19-111, IPA19-112, IPA19-113, IPA19-114) in Randomized Block Design at the New Research Farm, ICAR-IIPR, Kanpur. Morphological, biochemical/crop efficiency parameters and yield Characters were measured periodically. Anti-oxidant enzymes peroxidase and Super Oxide Dismutase activity was assayed as per standard methods. Conclusively, genotypes like NDA-2, MAL-13, ICP2275, IPACT-2, IPA19-106, IPA19-112 were highly tolerant to cold stress and genotypes Dholi- D, JBT 46/27, IPAD1-17, IPACT-22, IPA19-102 and IPA19-103 were highly susceptible to cold stress. Biochemical/plant efficiency parameters including NBI, Chlorophyll, flavonols, anthocyanin's, NDVI, Photosynthetic efficiency, antioxidant enzyme activities and finally test weight and grain yield were superior in tolerant group over susceptible group.

Conflict of interest

All authors declare that they have no conflicts of interest.

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