Indian Journal of Agricultural Sciences **93** (9): 978–983, September 2023/Article
https://doi.org/10.56093/ijas.v93i9.138073

Identification and characterization of photo-thermo insensitive cowpea (*Vigna unguiculata*) genotypes for hot arid environment

AJAY KUMAR VERMA[1]*, D K SAMADIA[1], HANUMAN RAM[1], CHET RAM[1], GANGADHARA K[2] and P S GURJAR[1]

ICAR-Central Institute for Arid Horticulture, Bikaner, Rajasthan 334 006, India

Received: 20 June 2023; Accepted: 08 August 2023

ABSTRACT

High green pod yield, dual purpose type, earliness and photo-thermo insensitivity are the major breeding objectives of cowpea [*Vigna unguiculata* (L.) Walp.]. Photo-thermo sensitivity makes this crop vulnerable to photoperiod and temperature fluctuations particularly in hot arid regions, thereby affecting its yield potential drastically. Therefore, the present study was aimed to identify such elite genotypes with photo-thermo insensitivity, which can fit well across all seasons under hot arid environment. The experiments were conducted at ICAR-Central Institute for Arid Horticulture, Bikaner during rainy and summer seasons of 2019–20 and 2020–21. A wide range of variability among genotypes was observed with respect to various phenological and yield traits over the environments. Among the evaluated genotypes, AHCP-1-4-1 and AHCP-2-3 were found photo-thermo insensitive as these were able to flower and set pods at temperatures as low as 10ºC and as high as 46°C. Pollen viability studies indicated that AHCP-1-4-1 had 83.72 and 88.24% pollen viability and AHCP-2-3 had 81.58 and 85.71% viable pollen at 11°C and 46°C, respectively and normal pollen tube growth at both the extremes of temperature. The identified genetic resources will contribute for developing photo-thermo insensitive cultivars and will improve the productivity and extend the availability of cowpea with quality pod yield across all seasons and locations of hot arid regions.

Keywords: Hot arid region, Molecular profiling, Photo-thermo insensitivity, Pollen viability

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important legume crop and a crucial part of farming systems throughout the tropics and warm subtopics. Green, immature cowpea pods are also known as black eye pea, southern pea, crowder pea, niebe caupi or frijole are used as vegetable. Numerous regional or vernacular names have been given to it, including Lobia (Hindi), Barbati (Bengali), Urohi (Assami), Sonta (Garhwali), Chavati (Marathi), Alasande (Kannada) and Manpayar (Malayalam). In general, arid legumes in India includes 4 minor legumes, viz. cluster bean (guar), moth bean, cowpea and horsegram. These legumes are known for drought tolerance, soil health sustainability and agriculture diversification and providing food, feed and various products of industrial and export value. In spite of such importance of these crops in regional, national and climatological context, their low productivity still needs to be improved (Kumar 2005).

Numerous biotic and abiotic stressors have an impact on the production and productivity of pulses (Sahoo *et al.* 2002). Non-availability of improved cultivars suitable for round the year cultivation with greater suitability and general adaptability is one of main reasons for the low yield (Samadia and Haldhar 2019). Further, the majority of the cowpea cultivars are appropriate for a particular agroclimatic zone and cropping season. Most of the time, the absence of photo-thermo insensitivity renders them susceptible to variations in photoperiod and temperature, greatly reducing their potential yield, particularly when the same variety is cultivated across many seasons (Pratap *et al.* 2014, Bhanu *et al.* 2017). The importance of photo-thermo insensitive trait in cowpea has also been emphasized by Kumar (2005) for their use for longer sowing period and harvesting. Though, some photo-insensitive varieties have been developed in the country, they are unable to express their potential in hot arid regions due to their sensitivity to extremes of temperature. Therefore, there is a need to identify the sources/donors for photo-thermo insensitivity trait which can be utilized in cowpea breeding programmes. In view of the above, the present study was aimed to identify photo-thermo insensitive genotypes of cowpea with horticultural traits suitable for hot arid environment.

MATERIALS AND METHODS

The field experiment was conducted at research farm

[1]ICAR-Central Institute for Arid Horticulture, Bikaner, Rajasthan; [2]Central Horticulture Experiment Station, ICAR-Central Institute for Arid Horticulture, Vejalpur, Panchmahal, Gujarat. *Corresponding author email: ajayhorti19@gmail.com

of ICAR-Central Institute for Arid Horticulture, Beechwal, Bikaner (28°N, 73° 18° E at an altitude of 234.84 m amsl), Rajasthan during rainy and summer seasons of 2019–20 and 2020–21. The 6 cowpea genotypes, viz. Arka Garima, Pusa Komal, Kashi Nidhi, Kashi Kanchan, AHCP-1-4-1 and AHCP-2-3 were evaluated under natural field conditions. For rainy (*kharif*) season evaluation, the seed material was sown on 01st August during both the years. Similarly, for summer season evaluation, the seed material was sown on 15th February during both the years. Each genotype was sown under drip system of crop cultivation having row to row distance of 60 cm and plant to plant distance of 45 cm. Standard package of practices recommended for cowpea cultivation was followed to raise a good crop. Weekly weather data on maximum and minimum temperatures (°C) and relative humidity during crop period of both the years (August, 2019–June, 2020 and August, 2020–June, 2021) were obtained from the meteorological observatory of the Agricultural Research Station, Beechwal, Bikaner. All the individual rows of all the genotypes were visited and observed every day to record the dates of important phenological events, viz. days to first flower, days to 50% flower, days to first pod harvest, days to last pod harvest, number of flowers/cluster, number of pods/cluster and green pod yield. Observations on days to first flower, days to 50% flower, days to first pod harvest, days to last pod harvest were recorded on whole row basis while, rest of the observations were observed on 5 random plants and were averaged.

Pollen viability studies: Viability of the fresh pollen samples in those genotypes which survived under variable daylength and extremes of temperature during rainy and summer seasons was determined based on their stainability which was done by acetocarmine technique as described by Roberts (1977). One hundred pollen grains were counted per slide with 10 slides each. The pollen grains which stained deeply and looked normal were considered as viable, whereas weakly stained were counted as non-viable (Pearson and Harney 1984) and the percentage of pollen viability (number of stained pollens/ total number of pollens × 100) was determined accordingly. Germination of fresh pollen grains was tested by sucrose-hanging-drop culture with minor modifications (Visser *et al.* 1977). The pollen tube germinated equal to or greater in diameter than the pollen grains was taken into account as pollen germination (Pearson and Harney 1984).

Molecular profiling: For molecular characterization of AHCP-1-4-1 and AHCP-2-3 genotypes, leaf samples were collected from field along with 19 genotypes as comparative controls and stored at deep freezer (-80°C) till DNA isolation. DNA was isolated from 100 mg of leaf tissue using DNeasy Plant Mini Kit (Qiagen, Netherlands) with some minor modifications. The quality and quantity of DNA was assessed electrophoretically on 0.8% agarose gel and by spectrophotometer (GeneQuant, Pharmacia, France), respectively. Twelve CBDP and 10 ScoT markers were selected from the studies of Singh *et al.* (2014) and Collard and Mackill (2009), respectively and custom synthesized by Eurofins Genomics, India Pvt. Ltd, Bengaluru. The PCR profiling of CBDP and ScoT markers was done in 20 ul of reaction cocktail containing 2.0 ul of 10X Taq DNA polymerase buffer, 0.5 ul of dNTP mix (10 mM), 1.0 ul of $MgCl_2$ (25 mM), 2.0 ul of primer (10 mM), 1U of Taq DNA polymerase enzyme (Genetix Biotech Asia Pvt. Ltd., India), 3.0 ul of template DNA (20 ng/ul), and final volume was made to 20 ul with nuclease- free water (Hi-media, India). For PCR amplification of CBDP markers, a touch-down PCR programme was adopted according to Singh *et al.* (2014). The thermal profile for ScoT markers was done as follows: Initial denaturation at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 30 sec, primer annealing at 45°C for 30 sec and extension at 72°C for 2 min. The final extension for both types of markers was done at 72°C for 10 min, followed by hold at 10°C. The PCR amplicons of CBDP and ScoT markers were analyzed electrophoretically on 1.2–1.5% agarose gel with 1 kb standard DNA ladder (Fermentas, USA). The specific bands to AHCP-1-4-1 and AHCP-2-3 genotypes were high-lighted as DNA fingerprints for identification of these genotypes to rest of the comparative controls.

RESULTS AND DISCUSSION

During the study period, wide variations were observed in the weather parameters for both the years (August, 2019–June, 2020 and August, 2020–June, 2021). Weekly maximum and minimum temperature along with relative humidity during the crop period is presented in Fig 1. All the weather parameters were variable and the weekly minimum and maximum temperatures during rainy season of both the years (2019–20 and 2020–21) ranged from 12.8 to 40.7 and 8.5–29.8°C, respectively. Similarly, during summer season of both the years, the weekly minimum and maximum temperatures ranged from 10.3 to 46.3 and 16.3 to 42.4°C, respectively. The lowest temperatures during rainy season of both the years were recorded in the month of November and the highest temperatures were recorded in the month of May.

Crop phenology is an important trait to know the favourable and sensitive stages of a crop plant to the particular environment. Large variations in days to flowering, days to 50% flowering, days to first and last tender pod harvest were observed among the evaluated genotypes during both the seasons and years (Table 1). During rainy season of 2019–20, days to first flowering, days to 50% flowering, days to first and last tender pod harvest ranged from 30–42, 33–46, 40–58 and 81–112 days after sowing (DAS), respectively. Similarly, during summer season of 2020–21, these ranged from 36–46, 41–51, 46–63 and 78–122 DAS, respectively. There was not much effect of daylength at moderate temperature on days to first and 50% flower in all the genotypes under study. However, the reproductive growth traits, viz. days to first and last pod harvest were influenced by shortened daylength when the temperature went below 25°C in genotypes, viz. Arka Garima, Pusa Komal, Kashi

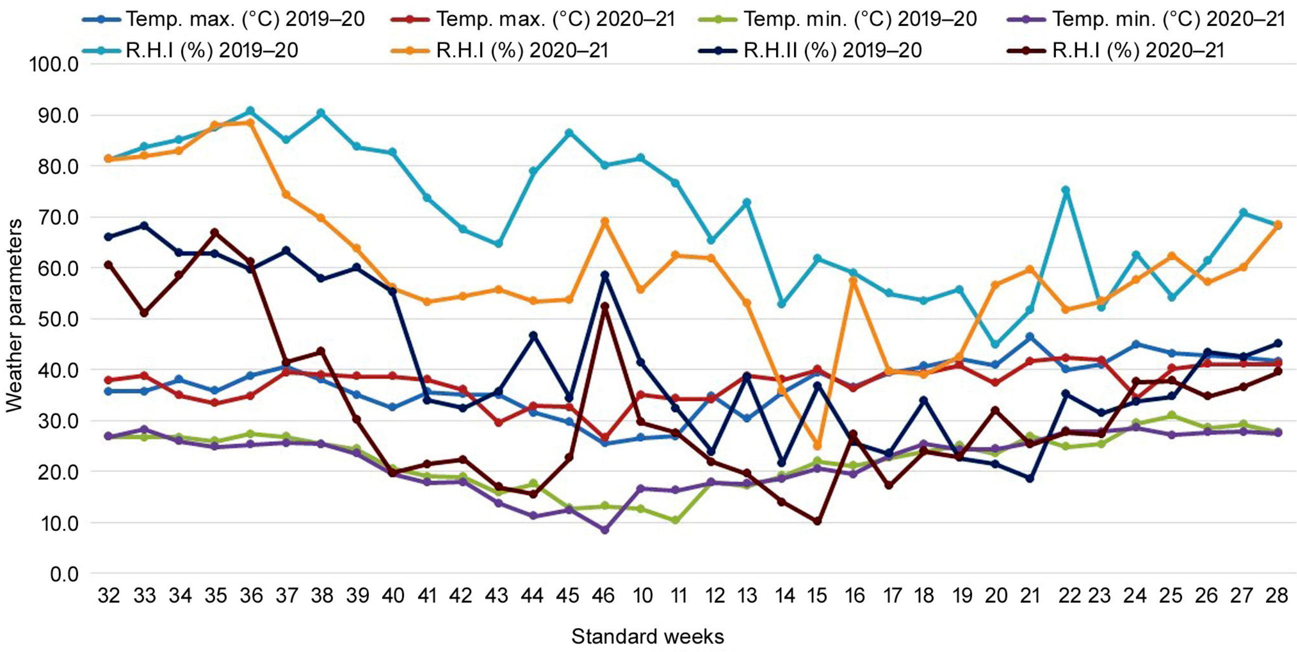

Fig 1 Weather parameters during crop period (August-June, 2019–20 and 2020–21).

Nidhi and Kashi Kanchan. In these genotypes, days to last pod harvest ranged between 78–92 DAS during both the seasons and years. These genotypes were not able to set pods after 44–46th standard week when the minimum temperature dropped below 10^{o}C during rainy season of both the years. Similarly, these genotypes were not able to set pods after 20–24th standard week when the maximum temperature raised above 41^{o}C. The similar effects of photoperiod and temperature on phenological cycle of cowpea has also been reported by Barros *et al.* (2021). The seasonal differences for these traits between rainy and summer season might be due to effect of photoperiod and temperature under varying daylength conditions (Zhang *et al.* 2020). In contrast to this, reproductive growth of cowpea genotypes AHCP1-4-1 (IC-0625644) and AHCP-2-3 (IC-0628910) was not influenced by decreasing temperature and variable daylength as these genotypes had last pod harvest up to 122 DAS and had longer podding duration than rest of the genotypes under study. These two genotypes had normal reproductive behaviour under both rainy and summer seasons and were able to set pods at temperature as low as 10^{o}C and as high as 46^{o}C (Verma *et al.* 2022). The genotypes which were able to set the pods at low temperature (during the cooler months of November) and also at high temperature (the warmer months of May-June) were considered as photo and thermo-insensitive. Thus, the genotypes, viz. AHCP-1-4-1 and AHCP-2-3 were found photo-thermo insensitive under hot arid environment. Such type of sources for photo-thermo insensitivity based on podding ability at varying temperatures in *Vigna* species have also been reported by Karim *et al.* (2003), Nuhu and Mukhtar (2013), Pratap *et al.* (2014) and Bhanu *et al.* (2017).

Observing normal reproductive behaviour both under low and high temperatures, pollen viability study was conducted in these genotypes in the 44th and 21st standard week of the year 2020 when the minimum and maximum temperature was 11°C and 46^{o}C, respectively. Both the genotypes had normal pollen behaviour and pollen tube germination (Fig 2). The genotype AHCP-1-4-1 had 83.72 and 88.24% pollen viability at 11°C and 46^{o}C temperature, respectively. Similarly, the genotype AHCP-2-3 had 81.58 and 85.71% pollen viability at 11 and 46^{o}C temperature, respectively. The percentage of pod set and green pod yield of AHCP-1-4-1 and AHCP-2-3 was also significantly higher than the other genotypes (Table 2). These results further support the photo-thermo insensitivity of these two genotypes as there was more than 81% pollen viability along with normal pollen tube germination. The results of higher percentage of pod set in photo-thermo insensitive cultivars than the sensitive cultivars in *Vigna* species corroborated with the findings of Karim *et al.* (2003), Angelotti *et al.* (2020) and Barros *et al.* (2021). Effect of photoperiod and temperature on pollen viability of *V. umbellata* and *V. glaberesens* was also observed by Pratap *et al.* (2012). Zhou *et al.* (2015) also reported the higher pollen germination and fruit set at high temperature in heat tolerant tomato lines than the heat susceptible ones. The sensitivity of pollen towards heat under high temperature ranges has also been documented by Rieu *et al.* (2017). Therefore, it is evident that the cowpea genotypes, viz. AHCP-1-4-1 and AHCP-2-3 have tolerance to changing photoperiods and temperature as suggested by profuse growth, good pollen viability, normal pollen tube germination and good pod set even at variable daylength and extremes of temperature.

Genotype-specific molecular markers help in varietal identification and maintenance of such genotypes in the ongoing crop improvement programmes (Gupta *et al.* 2013). For identifying the genotype-specific alleles, 10 ScoT and

Table 1 Flowering and pod harvest traits in cowpea genotypes over the environment

Genotype	Days to first flower				Days to 50% flower				Days to first pod harvest				Days to last pod harvest			
	RS	SS	RS	SS	RS	SS	RS	SS	RS	SS	RS	SS	RS	SS	RS	SS
	2019	2020	2020	2021	2019	2020	2020	2021	2019	2020	2020	2021	2019	2020	2020	2021
Arka Garima	40	46	42	45	44	51	46	51	56	62	58	63	88	87	91	89
Pusa Komal	32	37	33	38	35	42	36	44	43	53	47	56	81	78	83	79
Kashi Nidhi	35	40	34	38	37	45	39	43	50	54	49	53	89	87	85	88
Kashi Kanchan	38	42	39	44	41	47	43	49	51	55	50	56	90	88	92	91
AHCP-1-4-1	34	42	36	41	39	46	40	46	42	49	44	50	107	117	112	122
AHCP-2-3	30	38	34	36	33	42	37	41	40	47	41	46	105	110	108	113
SEm ±	1.68	1.71	1.23	1.81	1.28	1.43	1.49	0.17	2.45	2.26	1.60	2.62	3.87	3.27	4.16	3.36
LSD (P=0.05)	5.36	5.45	3.92	5.76	4.10	4.56	4.75	0.54	7.82	7.21	5.09	8.37	12.35	10.45	13.27	10.72

RS, Rainy season; SS, Summer season.

Table 2 Yield and its attributing traits in cowpea genotypes over the environment

Genotype	Flowers/cluster				Pods/cluster				Per cent pod set				Green pod yield (q/ha)			
	RS	SS	RS	SS	RS	SS	RS	SS	RS	SS	RS	SS	RS	SS	RS	SS
	2019	2020	2020	2021	2019	2020	2020	2021	2019	2020	2020	2021	2019	2020	2020	2021
Arka Garima	3.02	2.81	2.63	3.02	1.67	1.31	1.52	1.19	55.30	46.62	57.79	47.22	112.37	97.24	118.42	99.67
Pusa Komal	3.05	4.32	3.56	3.05	1.32	1.27	1.48	1.21	43.28	29.40	41.57	35.07	97.46	84.26	95.73	83.62
Kashi Nidhi	2.54	2.95	3.08	2.54	1.25	1.23	1.42	1.05	49.21	41.69	46.10	38.75	123.07	101.05	119.24	98.16
Kashi Kanchan	2.63	3.27	2.52	2.63	1.38	1.31	1.34	1.18	52.47	40.06	53.17	41.70	122.65	106.13	127.64	104.58
AHCP-1-4-1	5.41	4.89	5.91	5.41	4.32	3.78	4.71	4.02	79.85	77.30	79.70	78.52	172.38	169.82	178.14	175.83
AHCP-2-3	5.23	5.12	5.27	5.23	4.13	4.21	4.35	4.42	78.97	82.23	82.54	80.95	165.43	161.17	163.28	160.45
SEm ±	0.18	0.18	0.15	0.17	0.12	0.11	0.10	0.07	2.90	2.43	2.24	2.50	4.73	4.42	4.51	5.35
LSD (P=0.05)	0.59	0.56	0.49	0.53	0.37	0.35	0.31	0.23	9.27	7.75	7.16	7.98	15.08	14.10	14.41	17.06

RS, Rainy season; SS, Summer season.

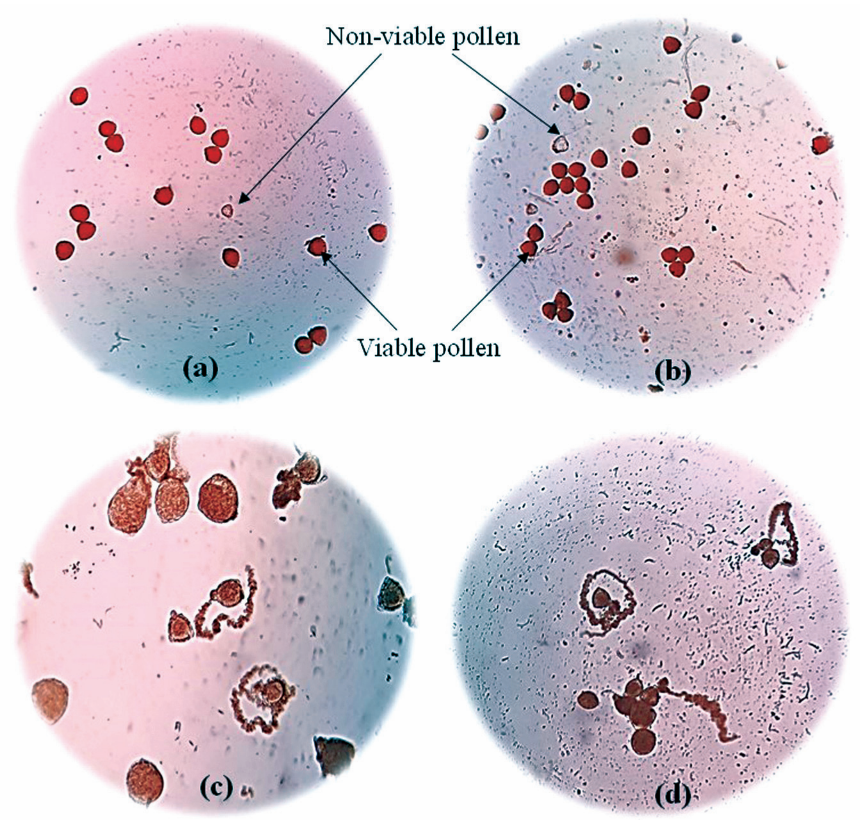

Fig 2 Pollen viability [AHCP-1-4-1 (a) and AHCP-2-3 (b)], and pollen tube germination in cowpea [AHCP-1-4-1 (c) and AHCP-2-3 (d)].

12 CBDP markers were profiled using PCR amplification on genomic DNA of 21 cowpea genotypes including two photo-thermo insensitive genotypes, viz. AHCP-1-4-1 (IC-0625644) and AHCP-2-3 (IC-0628910). For getting fidelity of the specific allele in AHCP-2-3 and AHCP-1-4-1 genotypes, 19 genotypes of cowpea including prominent varieties were taken as comparative control samples. The alleles common to all 21 analyzed cowpea genotypes were considered monomorphic and hence were not used as informative and specific allele to the AHCP-1-4-1 and AHCP-2-3 genotypes (Fig 3). On the other hand, the allele(s) which was amplified at variable position and only present in AHCP-1-4-1 and AHCP-2-3 genotypes were considered as specific alleles to distinguish these genotypes. Hence, the specific alleles to AHCP-1-4-1 and AHCP-2–3 genotypes are good enough to distinguish and identify these genotypes to rest of the analysed control samples. In this investigation, 5 ScoT markers namely ScoT1, ScoT17, ScoT18, ScoT19 and ScoT20 were amplified at least one to three specific alleles to the AHCP-2-3 and AHCP-1-4-1 genotypes. The ScoT19 has produced 3 specific alleles (approx. 400 and 650 and 1100 bp). Similarly, 5 CBDP markers produced at least one specific alleles to either in the AHCP-1-4-1 and AHCP-2-3 genotypes (Fig 3). Thus, the photo-thermo insensitive AHCP-2-3 and AHCP-1-4-1 genotypes are different from the comparative control samples based on these specific alleles.

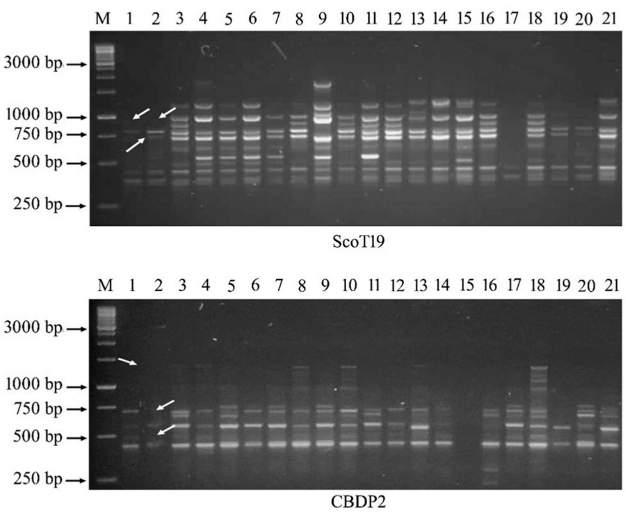

Fig 3 Molecular profiling of CBDP and ScoT markers for identification of AHCP-1-4-1 and AHCP-2-3 genotypes. (Numerical 1 and 2 indicate the AHCP-1-4-1 and AHCP-2-3 genotypes)

Thus, based on the study of crop phenology, yield traits, pollen viability and germination over the environment, it can be concluded that the cowpea genotypes AHCP-1-4-1 and AHCP-2-3 possesses unique trait of photo-thermo insensitivity under extremes of temperature (10–46^{o}C) which makes these genotypes invulnerable to photoperiod and temperature fluctuations particularly in hot arid regions. The identified genetic resources can be utilized in cowpea improvement programmes for incorporating photo-thermo insensitivity for developing improved cultivars. This will improve productivity and extend the availability of cowpea with quality pod yield across all seasons and locations under stressful environment of hot arid regions.

REFERENCES

Angelotti F, Barbosa L G, Barros J R A and Santos C A F D. 2020. Cowpea development under different temperatures and carbon dioxide concentrations. *Pesquisa Agropecuária Tropical* **50**.

Barros J R A, Guimaraes M J M, Silva R M E, Rêgo M T C, de Melo N F, de Melo Chaves, A R and Angelotti F. 2021. Selection of cowpea cultivars for high temperature tolerance: physiological, biochemical and yield aspects. *Physiology and Molecular Biology of Plants* **27**: 29–38.

Bhanu A N, Singh M N and Srivastava K. 2017. Identification of MYMV resistant and photo-thermo insensitive lines in mungbean. *Journal of Food Legumes* **30**(1): 20–24.

Collard B C Y and Mackill D J. 2009. Start codon targeted (SCoT) polymorphism: A simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Molecular Biology Reporter* **27**: 86–93.

Gupta S, Gupta D S, Anjum K T, Pratap A and Kumar J. 2013. Transferability of simple sequence repeat markers in blackgram. *Australian Journal of Crop Science* **7**: 345–53.

Nuhu Y and Mukhtar FB. 2013. Screening of some cowpea genotypes for photosensitivity. *Bayero Journal of Pure and Applied Sciences* **6**(2): 31–34.

Karim M A, Fukamachi H, Komori S, Ogawa K and Hidaka T. 2003. Growth, yield and photosynthetic activity of *Vigna radiata* L. grown at different temperature and light levels. *Plant Production Science* **6**(1): 43–49.

Kumar D. 2005. Status and direction of arid legumes research in India. *The Indian Journal of Agricultural Sciences* **75**(7): 375–91.

Pearson H M and Harney P M. 1984. Pollen viability in Rosa. *Hort Science* **19**: 710–11.

Pratap A, Gupta D S and Rajan N. 2012. Mungbean. *Breeding Indian Field Crops*, pp. 208–27. D Bharadwaj (Ed). Agrobios Publishers, New Delhi.

Pratap A, Basu P S, Gupta S, Malviya N, Rajan N, Tomar R, Madhavan L, Nadarajan N and Singh N P. 2014. Identification and characterization of sources for photo- and thermo-insensitivity in Vigna species. *Plant Breeding* **133**(6): 756–64.

Rieu I, Twell D and Firon N. 2017. Pollen development at high temperature: from acclimation to collapse. *Plant Physiology* **173**(4): 1967–76.

Roberts A V. 1977. Relationship between species in the genus Rosa, section Pimpinellifoliae. *Botanical Journal of the Linnean Society* **74**: 309–28.

Sahoo L, Sugla T and Jaiwal P K. 2002. *In vitro* regeneration and genetic transformation of *Vigna* species. *Biotechnology for the Improvement of Legumes*, pp. 1–40. P K Jaiwal and R P Singh (Eds.). Kluwer, The Netherlands.

Samadia D K and Haldhar S M. 2019. Scope and strategies for genetic improvement in vegetable crop-plants under high temperature and abiotic stressed climate of Rajasthan: A gap analysis. *Journal of Agriculture and Ecology* **8**: 1–18.

Singh A K, Rana M K, Singh S, Kumar S, Kumar R and Singh R. 2014. CAAT box-derived polymorphism (CBDP): A novel promoter-targeted molecular marker for plants. *Journal of Plant Biochemistry and Biotechnology* **23**: 175–83.

Verma A K, Samadia D K, Ram C, Ram H and Gangadhara K. 2022. Characterization and identification of photo-thermo insensitive genotypes of cowpea for climate resilience under hot arid environment. *(In) Abstracts of International Conference on AAFS*. pp. 389.

Visser T, De Vries D P, Welles G W H and Scheurink J A M. 1977. Hybrid Tea-rose pollen: I Germination and storage. *Euphytica* **26**: 721–28.

Zhang L X, Wei L I, Tsegaw M, Xin X U, Qi Y P, Sapey E, Liu L P, Wu T T, Shi S U and Han T F. 2020. Principles and practices of the photo-thermal adaptability improvement in soybean. *Journal of Integrative Agriculture* **19**(2): 295–310.

Zhou R, Yu X, Kjaer K H, Rosenqvist E, Ottosen C O and Wu Z. 2015. Screening and validation of tomato genotypes under heat stress using Fv/Fm to reveal the physiological mechanism of heat tolerance. *Environmental and Experimental Botany* **118**: 1–11.