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Research Article

Development and evaluation of early maturing thermo-tolerant Indian mustard (*Brassica juncea* L. Czern & Coss) genotypes for cultivation in semi-arid region of India

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Abstract

Heat stress at the seedling stage can increase mortality and has become an increasing threat for Indian mustard cultivation. Indian mustard is being predominantly grown under a rainfed ecosystem. In this context, the selection of heat tolerant genotypes may be one of the panaceas. Hence, this study was planned to evolve, evaluate and identify some of the promising early maturing heat stress tolerant from a pool of advanced breeding lines, to be used as probable donors for transferring heat tolerance. For this purpose, heat stress was imposed on 10 Indian mustard advanced breeding lines at the seedling period. The mean performance of the ten advanced breeding lines of Indian mustard with respect to morphophysiology revealed that the genotype DRMRHT-13-13-5-4 recorded earliest in maturity (117.5 days), high oil content (43.14 %) and seed yield of 3182.82 (kg/ha) which was 19.75 per cent higher seed yield over best check NPJ-112 under heat stress conditions. Similarly, the genotype DRMRHT-13-13-5-6 also recorded early in maturity (118.5 days), oil content (42.18%) and seed yield of 2964.92 (kg/ha) which was 11.55 per cent higher seed yield over the best check NPJ-112. There was a positive correlation between relative water content and membrane stability index ($r=0.945$) but both of these traits were negatively associated with excised-leaf water loss. Cluster survey classified 10 advanced breeding lines in five divergent groups. The tree diagram revealed abundant diversity between the Indian mustard genotypes for different characters and some magnitude of a consortium between different clusters. Based on the higher membrane stability index, water retention capacity of leaves, relative water content, minimum excised-leaf water loss and seed yield, advanced breeding lines DRMRHT-13-13-5-4 and DRMRHT-13-13-5-6 were identified as early in maturity and heat tolerant at a seedling stage which could be used in breeding programmes.

Key words

Indian mustard, Evaluation, Diversity, Early maturing, Thermo-tolerance, Physiological traits, Seed yield

INTRODUCTION

Brassica juncea is the predominant oilseed brassica in the Indian subcontinent. It accounts for about 80 per cent of the total area under rapeseed-mustard cultivation in India (Ram *et al.*, 2014, 2017). Rajasthan is among the largest producers, accounting for about 50 per cent of the total rapeseed-mustard production in India. The growing of rapeseed-mustard in Rajasthan is mostly carried out under conserve soil moisture conditions where sowing commences after southwest monsoon rains. Early

rains may lead the farmers to sow the crop early in the season to take advantage of conserved moisture in the soil (Venkateswarlu and Prasad, 2012). However, at the time of early sowing (second fortnight of September to the first fortnight of October), the mean surface soil temperature may reach as high as 45°C. High soil temperature often results in seedling mortality upon initial germination which may eventually require re-sowing (Salisbury and Gurung, 2011). A recent report by IITM,

Pune, Maharashtra indicates that annual mean surface temperature would rise from 3 to 5°C by the end of this century with warming more pronounced in north-western India. Therefore, efforts to strengthen resilience by genetic upscaling of heat stress at seedling and terminal stage in Indian mustard would be vital to stabilize the productivity of the crop in India. From the very beginning of agriculture, natural genetic variability has been exploited within crop species to meet subsistence food for the growing population (Govindaraj *et al.*, 2015). Evaluation of genetic diversity in Indian mustard using phenotypic characters has previously been reported by many researchers (Gupta *et al.*, 1991; Vaishnava *et al.*, 2006; Alie *et al.*, 2009; Singh *et al.*, 2010; Vinu *et al.*, 2013).

Angadi *et al.* (2000) attempted to decide the most sensitive crop growth stage for elevated temperature stress in three oilseed *Brassica* species, the differential in critical temperature among the *Brassica* species, and to estimate whether canola quality *Brassica juncea* is more tolerant to elevated temperature than other *Brassica* species. *Brassica juncea* has been identified as a substitute crop to canola as it exhibits considerable tolerance to heat and water stress. (Niknam and Turner, 1999; Wright *et al.*, 1996; Kirk and Oram, 1978; Parker, 1999) also reported Indian mustard to possess several agronomic advantages over Canola. However, considerable research on heat stress tolerance at the seedling stage in Indian mustard is yet to be conducted.

Identification of a gene, or genes responsible for the desired characteristics of heat tolerance at distinct stages of plant growth and development is of great importance. Consequently, the present investigation was come up with to develop, evaluate and identify some of the promising early maturing heat stress tolerant from amongst a pool of advanced breeding lines, to be used as potential donors for transferring heat stress tolerance in high yielding varieties.

MATERIALS AND METHODS

Indian mustard is sensitive to elevated temperature, especially at the seedling stage, which causes yield losses. To identify heat-tolerant genotypes of Indian mustard, ten advanced breeding lines including two checks, were grown in the field under heat stress conditions (maximum temperature 44.1°C at 0 to 10 cm depths on seeding date on September 28, 2017) in CRBD with 3 replications at the ICAR-DRMR, Bharatpur, Rajasthan, India. Geographically, the experimental farm of ICAR-DRMR, Bharatpur is situated at the altitude of 178.37 MSL, 77.27°E longitude and 27.12°N latitude. The area has a semi-arid, sub-tropical climate having mean precipitation of about 664 mm most of which is received in the rainy season spreading from July to September. The soil of the experimental site was sandy loam with EC 1.5 dSm⁻¹, organic carbon (0.25 - 0.30%), available N (125-135 kg/ha), P (20-22 kg/ha), K of 240-260 kg/ha, and

pH of 8.1. The crop was raised strictly under conserved moisture conditions. All genotypes were grown in five rows of five meter length. Row to row and plant to plant spacing was maintained at 45 and 15 cm, respectively. Recommended package of practices was followed to raise a good crop.

Morphophysiological characters viz., days to 50 per cent flowering (DTF), days to maturity (DTM), 1000- seed weight (1000 SW in g), Membrane Stability Index (PMSI in %), Excised-leaf water loss (PELWL in %), Relative water content (PRWC in %), Water retention capacity of leaves (PWRCL %), Oil content (POC %), Oil yield (OY in kg/ha) and Seed yield (SY in kg/ha) were recorded from five randomly selected plants of each genotype. Various physiological characters including PMSI, PELWL, PRWC and PWRCL were determined by the procedures described by Ram *et al.* (2015). The oil content was assessed by means of nuclear magnetic resonance (NMR) following the procedure described by AOCS (1980). The average seed weight/plant was calculated after threshing and weighing all mature siliquae from five arbitrarily designated plants.

Analysis of variance (ANOVA) was calculated according to the formula described by Panse and Sukhatme (1978) and critical differences (CD) were determined at 5 and 1% probability level. Estimation of phenotypic and genotypic coefficient of variation, heritability in the broad sense, genetic gain, and the correlation coefficient between seed yield and physiological parameters were determined using Windostat version 8.5 software. The studies on genotype coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) values superior to 20 per cent are considered as high while values less than 10 per cent are regarded to be low and values between 10 to 20 per cent to be intermediate (Deshmukh *et al.*, 1986).

Using the same morphophysiological data, Manhattan dissimilarity coefficients (Sokal and Michener, 1958) were calculated by pair-wise comparisons of varieties by using NTSYS-pc 2.02 programme (Rohlf, 1998). Based on an average linkage algorithm (UPGMA, unweighted pair group method with an arithmetic average), clustering of advanced breeding lines was done by means of agromorphological and physiological characters associated with heat stress tolerance using DARwin 6.0.14 software (Perrier and Jacquemoud-Collet, 2006).

RESULTS AND DISCUSSION

A total of eight advanced breeding lines was screened for earliness as well as physiological and yield contributing traits under heat stress conditions. The analysis of variance of the selected lines revealed a high significant difference among the genotypes for all the traits studied, an indirectly adequate quantity of genetic variability in the genetic material.

Table 1. Morpho-physiological characterization of advanced early and heat stress tolerant breeding lines of Indian mustard

| Genotype | Pedigree | DTF | DTM | 1000 SW | PMSI | PELWL | PRWC | PWRCL | POC | OY | SY |
|------------------------------|---|------|-------|---------|-------|-------|-------|-------|-------|---------|---------|
| DRMRHT-13-13-6-5GM2XBPR549-9 | | 41 | 115.5 | 5.63 | 9.92 | 50.00 | 84.35 | 58.81 | 42.41 | 1264.89 | 3054.79 |
| DRMRHT-13-13-5-6GM2XBPR549-9 | | 39.5 | 118.5 | 5.19 | 20.70 | 48.17 | 87.48 | 66.56 | 42.18 | 1250.83 | 2964.92 |
| DRMRHT-13-13-5-4GM2XBPR549-9 | | 38 | 117.5 | 5.66 | 23.51 | 48.01 | 84.45 | 65.90 | 43.14 | 1350.20 | 3132.82 |
| DRMRHT-13-13-5-5GM2XBPR549-9 | | 44.5 | 117 | 4.97 | 17.72 | 73.36 | 83.85 | 63.19 | 42.22 | 1282.02 | 3026.03 |
| DRMRHT-13-22-9 | JN032XBPR549-9 | 46 | 119.5 | 4.59 | 8.73 | 54.67 | 81.74 | 60.44 | 42.28 | 1302.55 | 3080.47 |
| DRMRHT-13-22-10 | JN032XBPR549-9 | 48.5 | 121.5 | 5.47 | 12.27 | 69.15 | 81.22 | 57.59 | 42.64 | 1494.00 | 3501.21 |
| DRMRHT-13-28-8 | BPR 543-2X BPR549-9 | 50 | 120 | 7.5 | | | | | 42.81 | 1509.67 | 3528.87 |
| DRMRHT-13-28-16 | BPR 543-2X BPR549-9 | 48 | 119 | 6.75 | | | | | | | |
| | | | | | 14.56 | 56.65 | 80.45 | 59.22 | | | |
| | | | | | 10.65 | 51.90 | 78.94 | 63.43 | 41.89 | 1270.60 | 3033.07 |
| NPJ-112 (C) | SEJ 8 X Pusa Jagannat | 51.5 | 121.5 | 5.84 | | | | | 42.22 | 1122.25 | 2657.76 |
| | | | | | 16.97 | 54.53 | 76.08 | 61.92 | | | |
| BPR 543-2 (C) | GPre-registered for thermo-tolerance | 57 | 126 | 5.30 | | | | | 42.73 | 1256.54 | 2938.25 |
| | | | | | 15.85 | 56.81 | 79.12 | 60.39 | | | |
| CV (%) | | 2.40 | 3.98 | 4.02 | 5.02 | 6.27 | 6.91 | 4.11 | 2.63 | 5.26 | 15.18 |

DTF- Days to 50% flowering; DTM- Days to maturity; 1000 SW-1000- seed weight (g); PMSI- Membrane Stability Index (%); PELWL- Excised leaf- water loss (%); PRWC- Relative water content (%); PWRCL- Water retention capacity of leaves (%); POC- Oil content (%); OY- Oil yield (kg/ha); SY- Seed yield (kg/ha).

Momentous variation was experienced among the genotypes for days to 50 % flowering, the mean was 46.4 days and ranged from 38 to 57 days (**Table 1**). The genotype DRMRHT-13-13-5-4 was the earliest to attain 50 per cent flowering (38 days), whereas the genotype BPR-543-2 was found to be late in flowering (57 days). The genotype DRMRHT-13-13-5-4 recorded early in maturity (117.5 days) than the best check NPJ-112 (121.5 days). The grand mean of 1000-seed weight was established to be 5.96 g and ranged from 4.59 to 7.5 g. The genotype DRMRHT-13-28-8 recorded a maximum 1000-seed weight of 7.5 g, whereas the genotype DRMRHT-13-22-9 recorded a minimum 1000-seed weight of 4.59 g. The membrane stability index was ranged from 9.92 to 23.51 per cent. There was significant variation in membrane stability index among various genotypes. The genotype DRMRHT-13-13-5-4 recorded maximum membrane stability index (23.51 %), while the genotype DRMRHT-13-22-9 attained the least membrane stability index (8.73%). Heat tolerance generally recovers membrane stability under heat stress situation. In general, cell membrane thermostability is an unbiased index of genetic variation in heat stress tolerance that stands for a rational association with plant performance under heat stress. The genotype DRMRHT-13-13-5-4 recorded minimum excised leaf-water loss (48.01 %) followed by genotype DRMRHT-13-13-5-6 (48.17%). The relative water content (%) grand mean was found to be 81.76 per cent and ranged from 76.08 to 87.48. The DRMRHT-13-13-5-6 attained maximum relative water content (87.48 %) followed by genotype DRMRHT-13-13-5-4 (84.45%). The genotype DRMRHT-13-13-5-6 recorded the maximum

water retention capacity of leaves (66.56%) followed by DRMRHT-13-13-5-4 (65.90%). The grand mean of oil content was estimated to be 42.45 per cent and ranged from 41.89 to 43.14 per cent. This character exhibited the least variation among the genotypes. The genotype DRMRHT-13-13-5-4 recorded maximum oil content of 43.14 per cent followed by genotype DRMRHT-13-28-8 (42.82%). At the same time substantial variation was exhibited for oil yield. The grand mean of seed yield was estimated to be 3091.82(kg/ha). The DRMRHT-13-13-5-4 recorded a 17.87 per cent higher seed yield increase over best check NPJ-112 under heat stress conditions.

The genotypic and phenotypic coefficient of variation, heritability and genetic advance as per cent of the mean were projected for ten genotypes and results are well-found in **Table 2**. The genotypic coefficient of variation ranged from 0.93 to 28.33 per cent. The maximum genotypic coefficient of variation (GCV) was witnessed for membrane stability index (28.33%) followed by excised-leaf water loss (14.14%) whereas the lowest value for GCV was recorded for oil content (0.93%) followed by DTM (2.28%). The broad sense of heritability ranged from 7.95 to 96.44 per cent. The maximum heritability was recorded for days to 50% flowering (96.44%) followed by excised-leaf water loss (79.00%) while the lowest was observed in seed yield (7.95%) followed by oil content yield (21.52%). The value of genetic advance as per cent of mean ranged from 1.79 to 47.09. The maximum genetic advance as per cent of mean was recorded for membrane stability index (47.09) followed by excised-leaf water loss (25.89) while the lowest genetic advance

Table 2. Mean, standard error, range, coefficient of phenotypic (PCV) and genotypic variance (GCV), heritability in broad sense and genetic advance for morpho-physiological traits in Indian mustard

| Characters | Mean \pm SEM | CV (%) | Range | PCV (%) | GCV (%) | Heritability h^2 (%) | Genetic advance as % of Mean |
|------------|----------------------|--------|-----------------|---------|---------|------------------------|------------------------------|
| DTF | 46.4 \pm 0.74 | 2.40 | 38-57 | 12.75 | 12.52 | 96.44 | 25.33 |
| DTM | 119.60 \pm 1.02 | 3.98 | 115.5-126 | 2.62 | 2.28 | 76.05 | 4.10 |
| 1000 SW | 5.69 \pm 0.38 | 4.02 | 4.59-7.5 | 16.63 | 13.24 | 63.38 | 21.72 |
| PMSI | 16.14 \pm 2.09 | 5.02 | 8.73-23.51 | 35.11 | 28.33 | 65.11 | 47.09 |
| PELWL | 56.32 \pm 2.75 | 6.27 | 48.01-73.36 | 15.91 | 14.14 | 79.00 | 25.89 |
| PRWC | 81.76 \pm 4.75 | 6.91 | 76.08-87.48 | 7.36 | 4.57 | 38.57 | 5.85 |
| PWRCL | 61.74 \pm 4.87 | 4.11 | 57.59-66.56 | 9.63 | 6.75 | 49.06 | 9.74 |
| POC | 42.45 \pm 0.28 | 2.63 | 41.89-43.14 | 1.37 | 0.93 | 46.30 | 7.01 |
| OY | 1310.36 \pm 88.53 | 5.26 | 1122.25-1509.67 | 11.36 | 5.27 | 21.52 | 5.03 |
| SY | 3091.82 \pm 217.56 | 15.18 | 2657.76-3528.87 | 10.94 | 3.08 | 7.95 | 1.79 |

DTF- Days to 50% flowering; DTM- Days to maturity; 1000 SW-1000- seed weight (g); PMSI- Membrane Stability Index (%); PELWL- Excised leaf- water loss (%); PRWC- Relative water content (%); PWRCL- Water retention capacity of leaves (%); POC- Oil content (%); OY- Oil yield (kg/ha); SY- Seed yield (kg/ha).

as per cent of mean was recorded in seed yield (1.79) followed by oil yield (5.03). The PCV value for membrane stability index is high, DTF, oil yield and DTM, POC and relative water content are found to be low. The studies showed that the PCV was higher than the GCV for all the traits, representing the effect of environmental variance in the rest of the variance studied. Similar findings were reported by Ram *et al.* (2012), Ram *et al.* (2017), Yadav and Pandey (2018), Gupta *et al.* (2019) and Thapa *et al.* (2020).

The character membrane stability index with higher values of PCV has been reported by Ram *et al.* (2017). Ram *et al.* (2017) also found a similar result for excised leaf water loss. The trait viz., membrane stability index showed a high value of GCV in the present investigation. The highest GCV and PCV value were observed for membrane stability index expressing the presence of the wide extent of variability for this character.

High heritability coupled with genetic advance as per cent of mean was exhibited by excised leaf water loss. Similar observations were reported by Ram *et al.* (2017) and Tripathi *et al.* (2019). 1000 seed weight articulated high heritability accompanied by genetic advance as per cent of mean which is in accordance with genetic advance as per cent of mean. These same results have been reported by Ram *et al.* 2015, Roy *et al.* 2015, Ram *et al.* 2017 and Kumar *et al.* 2018 and Tripathi *et al.* 2019. Expression of high heritability accompanied with high genetic advance as per cent of mean by various traits representing the lesser influence of environment and presence of additive gene action. Hence, an amenable for simple selection. Genotypic and phenotypic associations were estimated for yield and morphophysiological characters in all probable

combinations. The significant coefficient of association among seed yield and morphophysiological characters ranged from 0.521 to 0.976 (**Table 3**). The correlation coefficient between seed yield and morphophysiological traits revealed that relative water content recorded a significant association with seed yield ($r=0.899$) at the genotype level. Similarly, the water retention capacity of leaves exhibited a significant positive association with seed yield ($r=0.759$) at the genotypic level. There was a positive relationship among PRWC and PMSI ($r=0.945$) however both of these characters were negatively associated with PELWL. A positive and significant correlation was obtained between relative water content and membrane stability index, representing the possibility of increasing PRWC of leaves and membrane stability index by enhancing the level of heat stress tolerance. Similar finding has been reported by Ram *et al.* 2012, Ram *et al.* 2014, Ram *et al.* 2017, Kumar *et al.* 2018 and Jat *et al.* 2019. Oil yield recorded a positive association with seed yield ($r=0.987$) The result is in accordance with that reported by Ram *et al.* 2015, Ram *et al.* 2017 and Jat *et al.* 2019. Ram *et al.* (2015) reported that the PRWC showed a significant negative correlation with PELWL ($r = -0.385$) under heat stress condition.

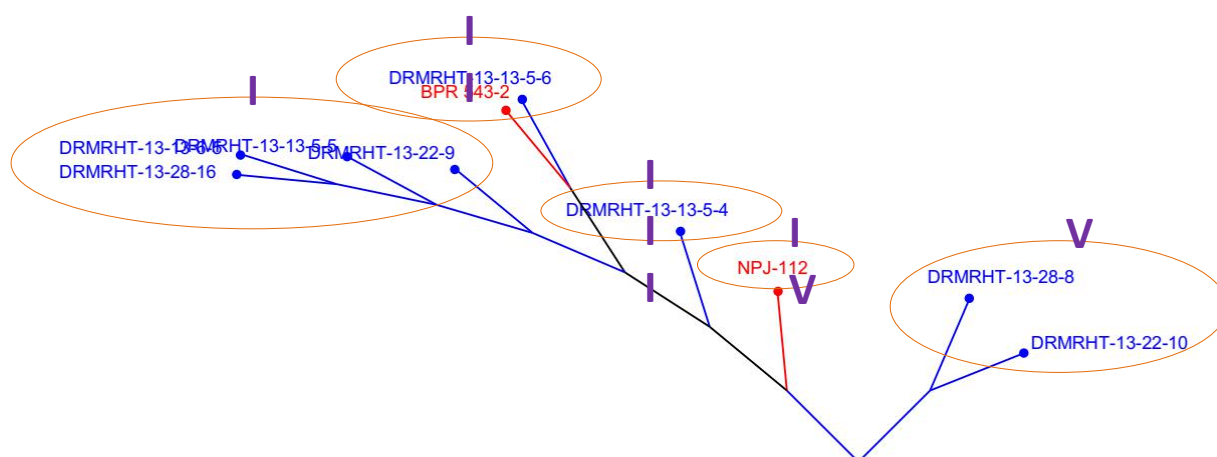
In UPGMA, the first cluster comprised four advanced breeding lines (**Fig.1**). The pedigree analysis of these four advanced breeding lines *i.e.* DRMRHT-13-13-6-5, DRMRHT-13-13-5-5, DRMRHT-13-28-16 and DRMRHT-13-28-16 revealed that BPR-549-9 one of the parents in all four lines are common. The advanced breeding lines of this cluster are characterized by moderately tolerant to heat stress and late in maturity. The second cluster had two advanced breeding lines. These advanced breeding lines are characterized by heat tolerant and higher

Table 3. Phenotypic (rp) and genotypic (rg) correlation coefficients between different morphological and physiological traits under heat stress situation in Indian mustard.

| Characters | | DTF | DTM | 1000 SW | PMSI | PELWL | PRWC | PWRCL | POC | OY | SY |
|------------|----|-------|---------|---------|--------|--------|----------|----------|--------|--------|---------|
| DTF | rp | 1.000 | 0.839** | 0.189 | -0.281 | 0.287 | -0.479 | -0.279 | 0.189 | -0.041 | -0.083 |
| | rg | 1.000 | 0.898** | 0.275 | -0.372 | 0.349 | -0.658* | -0.610* | 0.182 | 0.029 | -0.037 |
| DTM | rp | | 1.000 | 0.008 | 0.047 | 0.139 | -0.388 | -0.157 | 0.468 | -0.059 | -0.127 |
| | rg | | 1.000 | 0.021 | -0.130 | 0.166 | -0.015 | -0.363 | 0.433 | 0.139 | 0.101 |
| 1000 SW | rp | | | 1.000 | -0.070 | -0.155 | -0.051 | 0.135 | -0.040 | 0.364 | 0.368 |
| | rg | | | 1.000 | 0.079 | -0.264 | -0.849** | -0.499 | 0.364 | 0.430 | 0.407 |
| PMSI | rp | | | | 1.000 | -0.100 | 0.058 | 0.302 | 0.521* | -0.007 | 0.407 |
| | rg | | | | 1.000 | -0.141 | 0.945** | 0.955** | 0.586* | -0.454 | 0.066 |
| PELWL | rp | | | | | 1.000 | -0.028 | -0.180 | 0.097 | 0.226 | 0.221 |
| | rg | | | | | 1.000 | -0.610* | -0.886** | 0.223 | 0.620* | 0.976** |
| PRWC | rp | | | | | | 1.000 | -0.046 | -0.052 | 0.127 | 0.152 |
| | rg | | | | | | 1.000 | 0.642* | -0.101 | 0.089 | 0.899** |
| PWRCL | rp | | | | | | | 1.000 | -0.269 | -0.303 | -0.249 |
| | rg | | | | | | | 1.000 | -0.255 | 0.001 | 0.759** |
| POC | rp | | | | | | | | 1.000 | 0.308 | 0.168 |
| | rg | | | | | | | | 1.000 | 0.093 | 0.580* |
| OY | rp | | | | | | | | | 1.000 | 0.987** |
| | rg | | | | | | | | | 1.000 | 0.123 |
| SY | rp | | | | | | | | | | 1.000 |
| | rg | | | | | | | | | | 1.000 |

*, ** Significant at 5 and 1 per cent level of significance, respectively.

DTF- Days to 50% flowering; DTM- Days to maturity; 1000 SW-1000- seed weight ; PMSI- Membrane Stability Index; PELWL- Excised leaf- water loss; PRWC- Relative water content; PWRCL- Water retention capacity of leaves ; POC- Oil content ; OY- Oil yield); SY- Seed yield.

**Fig.1 UPGMA dendrogram depicting grouping of 8 early and heat tolerant advanced breeding lines along with checks**

yielder. DRMRHT-13-13-5-4 an advanced breeding line fell in cluster three of which pedigree is GM-2 x BPR-549-9. Advanced breeding line DRMRHT-13-13-5-4 is characterized by early maturity, recorded maximum MSI (23.51 %), more PWRCL (65.90), maximum POC (43.14) and seed yield (3132.32 kg/ha). The fourth cluster included only one advanced breeding line *i.e.* NPJ-112

which has a peculiar character as early in maturity, heat stress tolerance that put into a separate category. NPJ-112 is being used as a national check for early maturity and heat stress tolerant at the seedling stage. Two advanced breeding lines fell in cluster V of which one of the parent BPR-549-9 in their pedigree was common. DRMRHT-13-22-10 and DRMRHT-13-28-8 are characterized by the

medium in flowering and recorded maximum seed yield under heat stress condition. Similar finding has been reported by Vinu *et al.* (2013), Iqbal *et al.* (2014), Ram *et al.* (2015), Nagda *et al.* (2018) and Jat *et al.* (2019).

Based on higher PMSI, PWRCL, PRWC, minimum excised leaf water loss and seed yield, advanced breeding lines DRMRHT-13-13-5-4 and DRMRHT-13-13-5-6 were identified as early in maturity and heat tolerant at the seedling stage which could be used in breeding programmes.

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