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Physiological and Stress Studies of Different Rapeseed-Mustard Genotypes Under Terminal Heat Stress

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Abstract

Rapeseed-mustard constitutes an important source of edible oil next to soybean, grows under diverse agro ecological situations such as timely / late sown, rainfed / irrigated, sole-& / or mixed crop with cereals (wheat, barley etc.) and *rabi (October-April)* pulses (chickpea, lentil etc.), where high temperature is the main constraint not only at germination but also at grain filling stage. Flowering and grain filling are the most sensitive stages for temperature stress damage probably due to vulnerability during pollen and grain development, anthesis and fertilization leading to reduce crop yield. Effects of heat (high temperature) stress during terminal stage were investigated on photosynthesis, transpiration, stomatal conductance, stress intensity, mean productivity and geometric mean productivity , yield attributing characters and seed yield of 43 germplasm of rapeseed-mustard during 2010-11. Forty three rapeseed-mustard genotypes were sown in the field at two dates of sowing i.e., 1st optimum, D1 (Oct. 26, 2010) and 2nd in the last week of November, D2 (November 26, 2010) to allow the crop exposure to high temperature at terminal stage viz., grain filling stage. The crop sown in late November faced average maximum temperature of > 30^0C at grain filling stage. Genotypes were sown in augmented design. The stress intensity varied from 011 (BPR 349-9) to 0.82 (RH 304) While tolerance ranged from 1.05 (BPR 349-9) to 16.8 (EJ 17). Among the 43 genotypes, BPR349-9, PLM 2, RGN 197, RK 08-02 and BPR 549-9 had low stress intensity and tolerance. Terminal heat stress adversely effected photosynthesis and caused appreciable reduction from 9.8 (JS-29) to 48.3% (PBR-378) over the normal sown genotypes. The genotypes JS-29, KM-26, RMM-09-02 and PBR-357 showed < 15% reduction in photosynthesis under high temperature stress. The genotypes EJ-22, EJ-20, NPJ-124, NDT-05-02 showed less reduction in seed yield because of escape high temperature at terminal stage due to earliness. While the genotypes, BPR-549-9, BPR-540-6 and BPR-349-9 showed < 20% reduction in seed yield with high 1000-seed weight. All these genotypes showed superiority over both the checks (BPR 541-4 and JD-6). The study revealed that genotypes, BPR-549-9, BPR-540-6 and BPR-349-9 showed tolerant to high temperature at terminal stage based on less reduction in seed yield, low stress intensity, medium transpiration rate. Besides, this studies the other research done at DRMR, Bharatpur revealed superiority of these genetic stocks for high temperature tolerance at juvenile stage and salinity. On the basis of these studies BPR 349-9- was identified and got registered for high temperature tolerance at juvenile stage. BPR 540-6 (IC 0593927; INGR 13027)- for salinity and high temperature tolerance at juvenile stage. While BPR 549-9 (IC 0595525; INGR 13016)- was registered for salinity tolerance at juvenile stage and high water use efficiency. Thus these genotypes can be used to breed for high temperature tolerance.

Keywords- Stress intensity, stomatal conductance, water use efficiency, mustard

1. Introduction

One third of the world lands are classified as Arid and Semiarid Region and the remains are faced with water seasonal or local fluctuations. Aridity is the most common environmental stress and approximately include 25% of the world lands. Drought and high temperature stresses reduces leaf size, stem extension and root proliferation, disturbs plant water relations and reduces water-use efficiency. Usually, high temperature stress has detrimental effects on many processes in plants, which include reducing photosynthesis, accumulation of dry matter, translocation of dry matter and stomatal exchanges that affect their growth stages. Rapeseed-mustard is the third important oilseed crop in the world after soybean (*Glycine max*) and palm (*Elaeis guineensis* Jacq.) oil. Among the seven edible oilseed cultivated in India, rapeseed-mustard (*Brassica* spp.) contributes 28.6% in the total production of oilseeds. In India, it is the second most important edible oilseed after groundnut sharing 27.8% in the India’s oilseed economy. The global production of rapeseed-mustard was around 63.1 million tones from an area of 34.2 million hectare in 2012-13. Globally, Indian account for 19.3 % and 11.1% of the world acreage and production.

Mustard is cultivated in mostly under temperate climates. It is also grown in certain tropical and subtropical regions as a cold weather crop. Generally, plants respond to high temperature stress through developmental, biochemical and physiological changes and the type of the observed response depends on several factors such as stress intensity (SI), stress duration and genotype (Moradshahi et al., 2004). Rapeseed-mustard grows under diverse agro ecological situations such as timely / late sown, rainfed / irrigated, sole-& / or mixed crop with cereals (wheat, barley etc.) and *rabi (October-April)* pulses (chickpea, lentil etc.), where high temperature is the main constraint not only at germination but also at grain filling stage. Flowering and grain filling are the most sensitive stages for temperature stress damage probably due to vulnerability during pollen and grain development, anthesis and fertilization leading to reduce crop yield. High temperature in *Brassica* enhanced plant development and caused flower abortion and poor grain filling with appreciable loss in seed yield. A rise of 3^0C in maximum daily temperature (21-24^0C) during flowering and grain filling caused a decline of 430 kg / ha in canola seed yield. Therefore, improving seed yield of Indian mustard under late sown conditions is main challenge for rapeseed mustard research. Growing Indian mustard under late sown condition in northern India is getting importance under multiple cropping system. Hence there is need to develop terminal heat tolerant genotype on the basis of desirable physiological traits.

2. Materials and Methods

Site description and soil type.

This study was undertaken at the experimental farm of Directorate of Rapeseed-Mustard Research, Sewar, Bharapur (Rajasthan), India (latitude 27°15′ N, longitude 77°30′ E, elevation 178.37 m above mean sea level) during the periods 2010-11. This region has a sub-tropical and semi-arid climate (700 mm annual rainfall). The mean maximum temperature ranges from 18 to 24^0C and mean minimum temperature from 4 to 12.5^0C during crop season (November-March).The soil of the experimental site was a sandy loam and low in organic matter and alkaline in reaction. The soil is also low in phosphorus, zinc, iron and manganese by 73, 70, 19 and 8 percent, respectively.

Agronomic practices.

Experimental materials for the present study consisted of 43 advanced breeding lines/ varieties i.e., DRMR 802, PBR 331, NRCDR 601, RH 0216, NPJ 113, RB 50, NPJ 117, RH 0447, PLM 2, RH 0305, NRCDR 701, RH 0116, NRCDR 02, RB 55, RH 8814, DRMR 537-40, RGN 73, PBR 330, RGN 197, BPR 349-9, BPR 549-9, RH 555A, BPR 540-6, PBR 357, Parasmani 02-10, RRN 631, RK 08-02, HUJM 05-03, BPR 543-2, SKM 229, BAUM 2007, RH 0304, SKM 526, CS 54, RH 406, SKM 301, PRKS 28, BPR 541-4, JMMR 08-03, RGN 236, DRMR 541-44, RH 9615, PR 001 with four checks of Brassica juncea with four checks,EJ-17,NPJ 112,JD-6 and Sej-2 were sown in a augmented design during *rabi* season of 2010-2011 under normal (October 26, 2010) and late (November 26, 2010) sown condition to expose the seed development stage of genotypes to high temperature. The experiment sown late on November faced heat stress (>30^0C) during terminal stage. There were 5 rows of 5 m length for each genotype in a block. The row spacing was 30 cm and plant spacing with-in-a row was maintained at 10 cm by thinning. A fertilizer dose of 40: 40: 40 kg/ha (N: P_2O_5: K_2O) was applied at the time of sowing and 40 kg/ha N was top dressed 3-4 days after first irrigation. The two irrigations first at 35 DAS and second at 65 DAS and the soil moisture content was recorded at regular interval of 15 days at different soil depth.To protect the crop from the menace of aphid (*Lypaphis erysimi*) infestation, monochrotophos 36 % SL was sprayed @ 0.05% in 1000 litres water/ha in the first week of January. Final harvests were carried out in the month of March, 2012.

Estimation of traits.

Photosynthesis, transpiration, internal CO_2 concentration and stomatal conductance were recorded on 3rd and 4th fully expanded leaf from the top of five randomly taken and tagged plants in each replication with the help of portable photosynthesis system (CIRAS-2) at Bolting (65 DAS) and Flowering (75-80 DAS) stage. Water use efficiency (WUE) was computed as the ratio of photosynthesis to transpiration and expressed in µmoles/mmole. Stress intensity was calculated by using the formula: 1-Ys/Yn, where Ys is the yield under stress Yn is the yield under normal condition. While tolerance was measured as Yn-Ys. At maturity, five plants were hand harvested randomly from each experimental unit and the following parameters were determined: plant height, number of branches per plant, number of siliquae per plant, and number of seeds per siliqua. Main stem length was measured as the plant height. Numbers of siliquae per plant and seeds per siliqua were counted from 50 randomly selected siliquae after hand threshing. The crop was harvested manually in each plot separately and tied into bundles. The bundles were left in the field for drying until constant weight (12% moisture content). The sun-dried bundles were weighed for their biological (aboveground) yields.

3. Results and Discussion

During our investigation, the combined analysis of variance showed that the seed yield and its components including main shoot length, number of primary and secondary branches, number of siliquae on main shoot, primary and secondary branches and number of seeds per siliqua were drastically affected by high temperature at terminal stage. In terms of the majority of the agronomic traits, there was a significant difference between the studied cultivars ($P < 0.01$).

Table 1. Range,mean and C.V of different physiological and yield attributing characters of different genotypes under normal and terminal stage high temperature stage

Characters	**Normal Condition**			**Terminal high Temperature**		
	Range	**Mean±S.Em**	**C.V**	**Range**	**Mean±S.Em**	**C.V**
Pl. height	142-276	219±4.2	9.9	106-213	181.1±3.7	10.4
Primary Branches	3.8-7.8	6.1±0.12	10.9	3.2-7.4	4.7±0.13	15.4
Sec.Branches	1.0-15	7.7±0.56	39.0	0-10.2	4.1±0.38	50
Pods on P.B	71.0-234.4	149.6±5.1	17.8	28-67.5	53.7±1.00	7.9

Pods on S.B	5.0-182.2	73.4±5.7	42.1	0-98.6	35.5±3.4	55.3
Main shoot length	65-78.6	74.1±0.5	3.8	48-79	68.0±0.90	7.2
Pods on MSL	41.4-61.0	51.2±0.67	7.2	5.84-69.8	45.7±1.45	11.9
Siliqua length	3.86-6.84	5.2±0.80	8.0	4.14-12.2	6.0±0.20	13.4
Seeds/siliqua	9-28.2	14.1±0.42	12.8	9.8-19.2	13.7±0.28	11
1000 seed wt.	3.2-7.7	4.8±0.13	14.4	1.78-5.44	3.6±0.12	16.3
Plant dry wt.	30.0-80.0	53.3±0.027	30.9	15.0-50.0	36.4±0.33	14.5
Seed yield	7.4-21.8	13.6±0.11	24.2	2.5-10.5	6.4±0.13	26.9
Photosynthesis	4.3-15.5	8.5±0.49	19.1	4.5-15.5	8.7±0.26	23.3
WUE	0.90-5.2	2.3±0.11	24.2	0.90-4.6	2.3±0.13	26.9
Stress Intensity	0.11-0.82	0.51±0.03	30.9			
Tolerance	1.1-17.0	7.3±0.58	42.7			

Usually, fluctuations of plant height are the most conspicuous characteristic of genetic conditions and environmental changes in most plants. In this experiment, the highest plant height (276 cm) was that of normal irrigation (I) treatment, while high temperature at terminal stage caused the plant height to decrease by 22.8 %. Reduction in the plant height due to terminal stage high temperature stress is probably related to decline in photosynthetic products as a result of soil moisture decrease which eventually causes the plant not to reach its genetic potential. Other researchers have also reported a significant decrease in the stem height of rapeseed cultivars under terminal stage high temperature conditions.

In this study, terminal stage high temperature resulted in the reduced number of branches, siliqua on different branches, seeds/siliquae and siliquae length. Significant genotypic differences for these characters were also observed in the experiment. Number of primary and secondary branches under normal condition varied from 3.8-7.8 and 1.0-15.0 while due to terminal stage high temperature after significant decrease it varies from 3.2- 7.4 and 0-10.2 respectively. Among the genotypes, PBR 331,RB-50, NPJ 113, NPJ 117,RGN 73, RGN 197,BPR 540-6 and RK 08-02 showed high number of primary and secondary branches over their best check. The desired number of branches in the unit of surface is closely related to the soil moisture regime during the plant growth period (Ardell et al., 2001). Reduced number of branches during the shortage of soil moisture has been reported earlier (Sadaqat et al., 2003; Naeemi et al., 2007).

The number of siliquae per plant is the most important component of the seed yield in rapeseed (Angadi et al., 2003). In this experiment, the largest number of siliquae 234 and 182 were obtained on primary and secondary branches per plant under the normal condition while the terminal stage high temperature caused 71% and 45.8% decreases in siliquae number of primary and secondary branches per plant. Genotypes, NPJ-113, RGN 197,RK 08-02, SKM-229 and BAUM-2007 showed higher siliqua number on primary and secondary branches per plant over the best check JD-6. Seeds per siliqua varies from 9-28.2 with mean value 14.9under normal condition while under terminal stage high temperature it varies from 9-19.2 with mean value 13.7. Moreover, the exposure to this stress in the flowering and siliqua formation stages resulted in a considerable reduction in the number of siliquae per plant through more severe flower and siliqua abscissions (Sinaki et al., 2007).

Usually, when water deficit stress is applied after the flowering stage,it causes the number of siliquae per plant to reduce by shortening the flowering period, the reproductive growth duration, and finally the infertility of some flowers and their abscission (Wright et al., 1996). Among the studied cultivars, NRCDR 601, PBR 300, RGN 197,HUJM-05-03, CS 54, SKM301, JMWR 08-03 and DRMR 541-44 showed higher number of seeds per siliqua then the best check JD-6. which was due to their genetic potentials. In terms of this trait, the cultivars showed different reactions to high temperature. In this regard, Chauhan et al. (2009) reported that under high temperature stress conditions, lines of heat-sensitive rapeseeds experienced a sharp drop in the number of siliquae; while, in heat-tolerant lines, the reduction was much less. Generally, the number of seeds per siliqua and the TSW are the constituting components of the seed yield in rapeseed (Angadi et al., 2003). It has been found that the ability of different rapeseed genotypes to form seeds inside siliquae is different and the number of seeds per siliqua is affected by genetic factors (Rao, Mendham, 1991). The reduction in siliquae on main shoot and seeds / siliqua could be due to floral sterility as temperature > 27^0 C has been reported to induce floral sterility in canola (Morrison and Stewart 2002) as well as development of flowers in to seedless parthenocarpic fruits &/or flower abortion on the stem due to high temperature (Young et al. 2004). Moreover, TSW is one of the most important determining factors of seed yield and the existence of large seeds that filled well, caused this yield to increase. The seed weight ranged from 3.2-7.7g under normal condition which reduced significantly to 1.8-5.4g. Results of the present experiment in this regard were consistent with those of the earlier researchers (Chauhan et al.,2009). Probably, heat stress through disrupting the plant photosynthesis, decreased assimilates synthesis which is necessary for seed filling, and consequently it resulted in seed shrinkage and weight loss. Based on the results RH 0447, PLM-2, NRCDR-701, RH-55, RH-8814,PBR-300,BPR-349-9,RH 555A,PBR-357,RRN-631,SKM-229, BPR 541-4 and RGN-236 had the higher seed weights over the best check JD-6.

Our results indicated several reductions in the number of siliquae per plant, the number of seeds per siliqua , and TSW due to heat stress which tended to decline in the seed yield from 21.8 g to 10.5g. This finding was consistent with the reports of Chauhan et al. (2009). Among the studied cultivars, RGN 197 gives the highest seed yield even more than the best check while the other genotypes, RH 0447,RH 8814, PLM-2,BPR 349-9 and BPR-549-9 were at par with the checks.

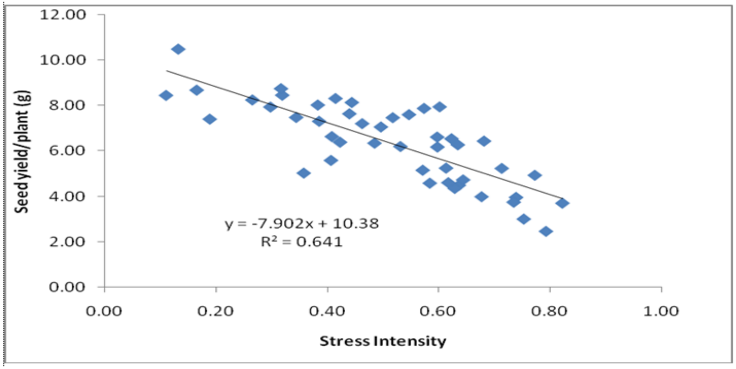

Fig.1. Relationship of seed yield with stress intensity under late sown condition

In general, the reaction of crops and their evaluation for an optimum yield under different environmental conditions depend on their ability to use the said conditions. This would be possible through regulating yield components and the interaction of genotype with the environment when desirable and undesirable conditions occur in each stage of plant growth and development (Entz, Flower, 1990).In this research, the highest biological yield was obtained from the normal condition(80g/plant), while heat stress led to a 37.5% decrease in the said trait. . Considerable reduction in seed yield under terminal stage high temperature in the present study could be due to less production of dry matter as a result of reduced LAI and CGR. The poor translocation of photosynthates from the sink to the source in the present study could be the other reasons for poor harvest index and consequently decreased seed yield in the present investigation. The results were in agreement with those of Subrahmanyam and Rathore (1994) who observed that high temperature during reproductive stage significantly inhibited the import of photosynthates by both upper and lower pods of terminal reaceme and thereby reduced sink strength.

The results indicated that there was a negative and highly significant correlation among seed yield and stress intensity as shown in Fig 1. The significant and negative correlation of stress intensity with seed yield ($r2$ =0.641, $P < 0.001$) showed that this criteria index was effective in identifying high yielding cultivars under high temperature conditions. Stress intensity has been found effective in identifying cultivars that perform well under stress conditions. A lower stress intensity value is an indicator of higher tolerance to high temperature stress. Based on this index, PLM-2, RGN-197, BPR-349-9, BPR 549-9 and RK 08-02 were identified as superlative cultivars in respect to terminal stage high temperature tolerance. The study further revealed positive and highly significant relationship of photosynthesis and water use efficiency with seed yield under normal and high temperature conditions. The stomatal conductance has negative relationship with water use efficiency.

4. Conclusion

The study further revealed positive and highly significant relationship of photosynthesis and water use efficiency with seed yield under normal and high temperature conditions. The stomatal conductance has negative relationship with water use efficiency. Hence the study revealed that genotypes, RGN 197,PLM-2BPR-549-9, BPR-540-6 and BPR-349-9 showed tolerant to high temperature at terminal stage based on less reduction in seed yield, low stress intensity, medium transpiration rate. Thus these genotypes were identified as superlative cultivars in respect to terminal stage high temperature tolerance.

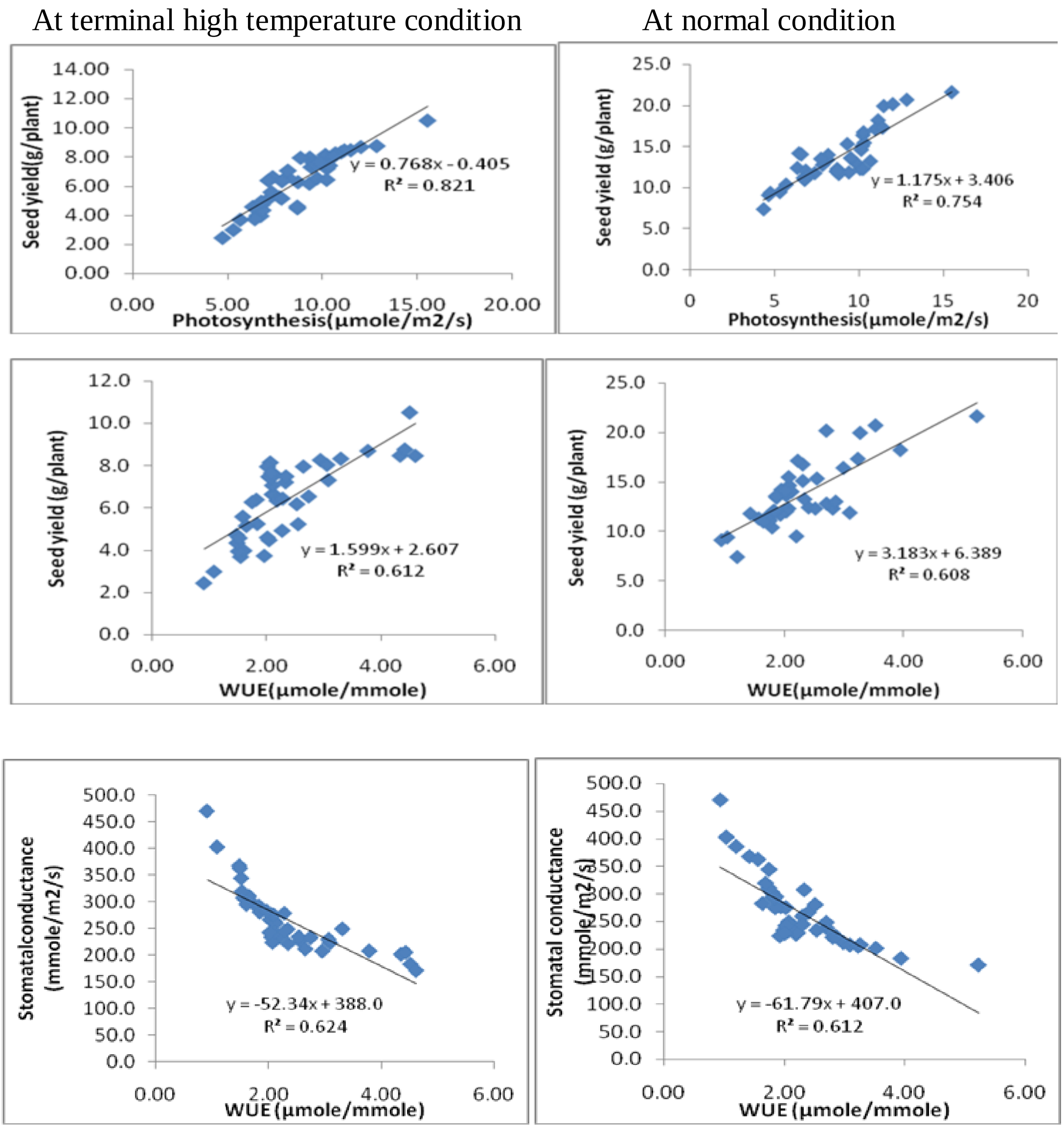

Fig. 2. The relationship between Seed yield and photosynthesis and water use efficiency and relationship between stomatal conductance with water use efficiency under normal and terminal stage high temperature condition

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