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SHORT COMMUNICATION

BIOCHEMICAL BASIS OF HIGH TEMPERATURE TOLERANCE DURING GERMINATION IN INDIAN MUSTARD (*BRASSICA JUNCEA* L.)

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The present investigation was carried out under laboratory conditions with 10 genotypes of Indian mustard (*Brassica juncea*) to study the high temperature induced changes during germination in carbohydrates, activities of peroxidase, superoxide dismutase (SOD), amylase and nitrate reductase enzymes. The temperature regime significantly affected all the characters studied. The genotypes x temperature interaction effects were significant only for germination and peroxidase activity. Increased temperature promoted the SOD and peroxidase but decreased nitrate reductase and amylase activities. Decline in the carbohydrates utilization in seeds limited by reduced amylase activity might have led to reduced germination. Germination exhibited positive and significant relationship with the activities of amylase (r = 0.392), nitrate reductase (r = 0.265*) and peroxidase (r = 0.506**) under high temperature regime. The regression analysis revealed that under high temperature regime, peroxidase and amylase enzyme activities were important contributors to germination accounting for 24.4 % and 14.5 %, respectively, of the total variability in seed germination.**

Keywords: **Amylase, antioxidant enzymes, *Brassica juncea,* germination, heat stress**

High temperature is the second most important stress next to drought, which can affect crop plants at any time and impose severe limitation on crop growth and development. The main adverse effects of this stress at the time of sowing are poor germination and seedling mortality resulting into poor crop stands and consequently seed yield. Allen (1995) reported that injury to plants due to heat stress might be the result of oxidative damage at cellular level. Further, hydroxyl radicals have been reported to cause damage to DNA, proteins, lipids, chlorophyll and almost every other organic constituent of the living cell (Becana et al. 1998). Alscher et al. (2002) suggested that anti oxidant enzymes such as peroxidase, catalase, superoxide dismutase provided possible defense mechanism to plants for protecting cellular and sub cellular system from the antitoxic effects of heat induced active oxygen radicals. Carbohydrate metabolism plays an important role in tolerance to heat stress (Volaire *et al.* 1998). High temperature caused lipid peroxidation and increased superoxide dismutase and peroxidase activity under high temperature in soybean (Zheng and Han 1997). Of late, high temperature at the time of crop establishment has been increasingly becoming an important impediment in rapeseed-mustard cultivation. The problem is further aggravated in rainfed production system where monsoon cessation based early sowing is crucial for higher productivity. Information regarding possible role of anti-oxidant enzymes and other biochemical characters under high temperature stress in Indian mustard is scanty. Therefore, the present investigation was carried out to study the high temperature induced changes during germination in

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carbohydrates, activities of peroxidase, superoxide dismutase, amylase and nitrate reductase enzymes.

The experiment was conducted in laboratory during July-October 2008. The experimental materials comprised 10 genotypes of Indian mustard (*Brassica juncea*), *viz*., BPR-349-9, BPR-540-6, BPR-542-14, BPR-543-2, GM-2, NPJ-92, NRCDR 02, PCR-7, Urvashi and Varuna. Twenty seeds from each genotype were placed in petri-dishes (9-cm diameter) lined with moist Whatman filter paper No. 42. There were two sets of experiments; one set was allowed to germinate in a BOD incubator at 25 $\pm 1^0$C (normal temperature) and 55-60 % relative humidity. The other set was exposed to 4 hours high temperature treatment (45 $\pm 1^0$C) during 11:00-15:00 hrs after 24 hrs of seed soaking and thereafter allowed to grow at 25 $\pm 1^0$C and 55-60 % relative humidity. The experiments were given 8 hrs photoperiod /day. Both the experiments were replicated thrice in completely randomized design. Germination was recorded when radicles of at least 2 mm length emerged and expressed in percentage. Twenty-four hrs after seed soaking, the carbohydrate content, and amylase activity was measured by the method of Yemm and Willis (1954) and Bernfeld (1955), respectively. The activities of peroxidase (POD), super oxide dismutase (SOD) and nitrate reductase (NR) were estimated following the methods of Palmiano and Juliano (1973), Dhindsa *et al.* (1981) and Jaworski (1971), respectively.

Analysis of variance was conducted considering the data in factorial completely randomized design to know the effect of genotypes, temperature regimes and their interactions on germination, carbohydrate content and enzymes. Simple correlations and regression analysis was conducted to study the relationship between antioxidant enzymes and germination as described by Gomez and Gomez (1984).

It indicated presence of variability among the genotypes investigated, as the sum of squares due to genotypes was highly significant for germination, super oxide dismutase, peroxidase, nitrate reductase and amylase. The temperature regime also significantly affected all the characters studied. The genotype x temperature interaction effects was significant only for germination and peroxidase activity implying that genotypes had differential response under different temperature regimes.

Genotype NRCDR 02 showed the highest germination (97.3%) followed by BPR-349-9 (96.5%) and Urvashi (94.4%) under high temperature regime (Table1). The high temperature decreased germination of all the genotypes significantly ranging from 1.4 (NRCDR 02) to 12 .9 % (PCR 7). Reduced germination in Indian mustard under high temperature has also been reported in earlier studies (Chhabra and Sharma, 2003 and Singh *et al.,* 2003). Variable response of genotypes in seed germination could be due to genotypic variation in temperature sensitivity of the embryo and protein synthesis as has been reported in maize (Riley, 1981).

Increased temperature promoted the activities of SOD and peroxidase (Table 2). Genotype BPR 543-2 had the highest SOD activity whereas; BPR 349-9 had the lowest activity. The high temperature induced increase in SOD activity varied from 14.0 (GM 2) to 31% (PCR 7) with a mean increase of 20.9 %. Peroxidaes activity varied from 81.4-113.8 units and 88.4-134.7 units under low and high temperature regimes (Table 2). The high activity of peroxidase under normal (BPR 543-2) and high temperature regime (NPJ 92) was also reflected in high germination of these genotypes (Table 1). Nevertheless, the high temperature invariably enhanced the activity by 16.6 %. The genotypic response in increase of peroxidase activity due to high temperature varied from 8.6 (BPR 540-6) to 27.1 % (NRCDR 2). High temperature induced increase in SOD and peroxidase activities were also reported in wheat (Sairam *et al.* 2000) and soybean (Zheng and Han 1997). Gupta and Gupta (2005) showed that the superoxide dismutase activity enhanced continuously with increasing temperatures during seedling growth in wheat as superoxide dismutase constitutes the first line of defense via detoxification of superoxide radicles. Increase in peroxidase activity under stress condition has been linked with protection from oxidative damage of lignification and cross-linking of cell wall (Dalal and Khanna-Chopra, 2001).

Table 1. Mean performance of genotypes for enzymes and carbohydrate content under normal and high temperature regimes.

Genotype	Germination (%)		SOD activity (Units/g fresh wt.)		Peroxidase activity (Units / g fresh wt)		Nitrate reductase (nmoles /hr/g)		Amylase activity (mg maltose/g / 20 minutes)		Total carbohydrate (mg/g fresh wt.)	
	25±1^{0}C	45±1^{0}C	25±1^{0}C	45±1^{0}C	25±1^{0}C	45±1^{0}C	25±1^{0}C	45±1^{0}C	25±1^{0}C	45±1^{0}C	25±1^{0}C	45±1^{0}C
BPR 349-9	98.6	96.5	685.7	795.2	87.6	96.5	14.4	11.1	141.3	132.7	2.98	4.32
BPR 540-6	93.8	88.5	722.5	921.3	81.4	88.4	10.7	8.3	135.1	123.2	3.41	4.38
BPR 542-14	96.0	86.0	766.5	988.9	82.9	92.4	18.5	11.2	134.3	121.5	3.31	4.20
BPR 543-2	94.5	89.2	957.6	1123.4	113.1	131.7	22.7	19.4	166.7	149.8	2.93	3.75
GM 2	91.4	86.4	785.3	894.6	89.3	108.6	12.2	9.5	143.6	132.2	3.54	4.19
NPJ 92	98.2	93.5	842.5	1021.5	108.4	134.7	21.1	16.9	152.5	133.0	3.41	4.06
NRCDR 2	98.9	97.3	890.5	1045.4	97.5	123.9	18.3	10.4	155.8	136.1	3.03	3.92
PCR 7	95.1	82.8	668.9	875.5	83.5	89.4	12.3	9.7	132.6	124.2	3.18	4.12
Urvashi	97.4	94.4	920.9	1061.8	98.6	123.1	17.3	14.9	146.4	129.2	3.61	4.12
Varuna	98.0	93.3	764.5	953.8	96.0	105.6	14.8	12.7	144.5	136.5	3.00	3.90
Mean ±	96.2	90.8	800.5	968.1	93.8	109.4	16.2	12.4	145.2	131.8	3.24	4.10
CD (P=0.05) for:												
Genotypes	3.2		46.5		7.6		2.1		9.0		NS	
Temperature	1.4		20.8		3.4		0.9		4.0		0.34	
Genotype x Temperature interaction	4.5		NS		10.8		NS		NS		NS	

* NS: Non-significant

Table 2. Effects of high temperature on germination and certain enzymes in Indian mustard.

Character	Range		Mean ± SEm		CV (%)	
	Normal temperature (25± 1^{0}C)	High temperature (45± 1^{0}C)	Normal temperature (25± 1^{0}C)	High temperature (45± 1^{0}C)	Normal temperature (25± 1^{0}C)	High temperature (45± 1^{0}C)
Germination (%)	91.4-98.9	80.7-97.3	96.2± 0.8	90.6± 1.7	2.6	5.9
SOD activity (Units/g fresh wt.)	608.9- 957.6	795.2 -1123.4	805.0 ± 31.3	971.5 ± 31.8	12.4	10.3
Peroxidase activity (Units/g fresh wt./min)	81.4-113.8	88.4 -134.7	93.8 ± 1.0	109.5 ± 1.3	11.6	16.3
Amylase activity (mg maltose/g fresh wt/ 20 minutes)	132.6 -165.7	121.5 – 149.8	145.2 ± 3.3	131.8 ± 2.6	7.2	6.3
Nitrate reductase (n moles/hr/g)	10.7- 22.7	8.3-19.4	16.2 ± 1.3	12.4 ± 1.1	24.6	28.8
Total carbohydrates (mg/g fresh wt.)	2.93- 3.61	3.75 - 4.28	3.24 ± 0.16	4.10 ± 0.14	7.7	4.8

Nitrate reductase, a key assimilatory enzyme responsible for reducing nitrate to nitrite which promotes seed germination by inhibiting H_2O_2 decomposition by anti oxidant enzymes like catalase (Hendricks and Taylorson 1974). The findings of the present investigation were consistent with these reports as the NR activity decreased because of high temperature by 39.9-43.2 % and could be the cause of substantially reduced germination in PCR 7, GM 2 and BPR 540-6.

Carbohydrates serve as important structural material and energy reserve for growth and are often associated with osmotic adjustment. Carbohydrate metabolism plays an important role in plant tolerance to various environmental stresses like drought and heat (Savin and Nicolas, 1996; Volaire *et al.* 1998). In the present investigation high temperature significantly reduced the amylase activity in all the genotypes. The amylase activity, in general, reduced by 10.1% with a range of 5.5(Varuna) –12.8 % (NPJ 92) under high temperature regime and total carbohydrates increased by 29.6%. The increase varied from 14.1(Urvashi) to 45.0% (BPR 349-9). The carbohydrate content ranged from 2.98 (BPR 349-9) to 3.61 mg /g fresh weight (Urvashi). Amylase activity showed a marked decline consequently leading to accumulation of carbohydrates under high temperature (Table 2).

The correlation analysis revealed that under normal temperature, seed germination was significantly and positively correlated with nitrate reductase activity (r = 0.392**) and peroxidase activity (r = 0.372**). Total carbohydrates were negatively associated with germination (r = -0.380**). But germination exhibited positive and significant relationship with the activities of amylase(r = 0.392**), nitrate reductase (r = 0.265*) and peroxidase (r = 0.506**) under high temperature regime. The regression analysis, however, revealed that nitrate reductase activity (Y = 0.244 X + 92.23) and total carbohydrates (Y = -3.774 X + 108.42) contributed 15.7% and 14.6 % to the variability in germination under normal temperature. But under high temperature regime, peroxidase and amylase enzyme activities were important contributors to germination accounting for 24.4 % and 14.5 %, respectively (Fig. 1a, b).

The results of the present study revealed that high temperature stress decreased germination by reducing the activities of nitrate reductase and amylase enzymes. Therefore, the carbohydrates utilization in seeds was

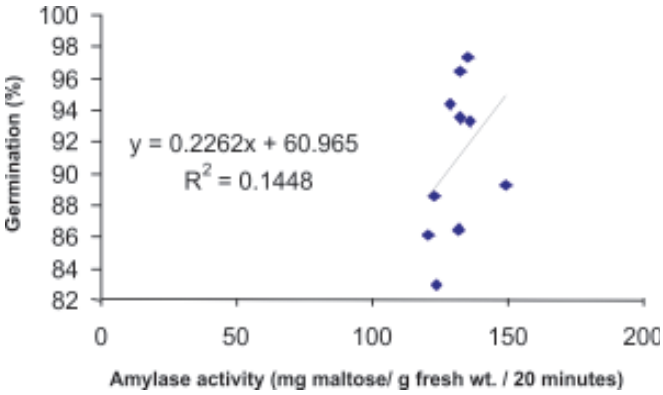

Fig.1a. Relationship between germination and amylase enzyme in Indian mustard under high temperature regime

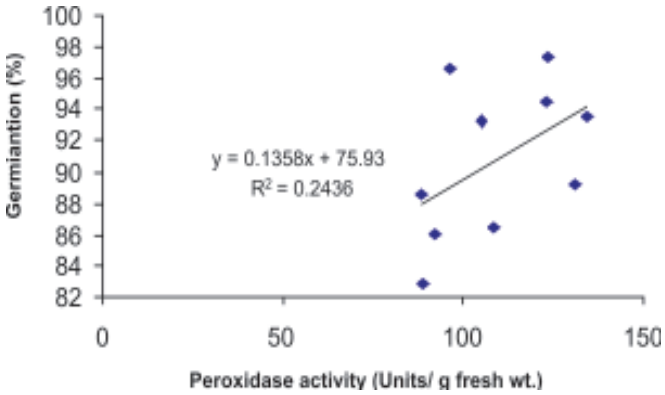

Fig.1b. Relationship between germination and peroxidase enzyme in Indian Mustard under high temperature regime

limited leading to reduced germination. Although, Indian mustard is an oilseed crop where fats could serve as an important source of food reserve to sustain growth but initial germination appeared to be dependent on the accumulated carbohydrates in the seed. Further, the activities of anti oxidant enzymes like peroxidase and SOD increased substantially due to high temperature stress, but peroxidase and amylase enzyme activities were important in germination.

REFERENCES

Allen, R.D. (1995). Dissection of oxidative stress tolerance using transgenic plants. *Plant Physiol*. **107**: 1049-1054.

Alscher, R.G., Erturk, N. and Heath, L.S. (2002). Role of superoxide dismutase (SOD) in controlling oxidative stress in plants. *J. Expt. Bot*. **53**: 133-141.

Becana, M., Moren, J.F. and Iturbe-Ormaetxe, I. (1998). Iron dependent oxygen free radicle generation in plants subjected to environmental stresses ; toxicity and antioxidant. *Plant and Soil* **201**: 137-147.

Bernfeld, P. (1955). Amylases, α and β. *Methods in Enzymology*. **1**: 149-158.

Chhabra, M.L. and Sharma, Rajesh (2003). Studies on the optimum temperature for germination in different genotypes of Indian mustard (*Brassica juncea* L.). pp.100.In. Abstracts. 11th Intl. Rapeseed Cong., Copenhagen, Denmark.

Dalal, M. and Khanna-Chopra, R. (2001). Differential response of antioxidant enzymes in leaves of necrotic wheat hybrids and their parents. *Physiol. Plant*. **111**: 297-304.

Dhindsa, R. S., Plumb-Dhindhsa, P. and Thorne, T. A. (1981). Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Expt. Bot*. **32**: 93-101.

Gomez, K. A. and Gomez, A. A. (1984). Statistical Procedures for Agricultural Research. 2nd Edition. John Wiley & Sons, New York. p. 680.

Gupta, Sunita and Gupta N. K. (2005). High temperature induced antioxidative defense mechanism in seedling of contrasting wheat genotypes. *Indian J. Plant Physiol*. **10**: 73-75.

Hendricks, S.B. and Taylorson, R. B. (1974). Promotion of seed germination by nitrate, nitrite hydroxylamine and ammonium salts. *Plant Physiol.* **54** :304-309.

Jaworski, K. (1971). Nirate reductase assay in intact plant tissues. *Biochem. Biophysics. Res. Comm*. **43**:1274-1279.

Palmiano, E. P. and Juliano, B.O.(1973). Changes in the activity of some hydrolases, peroxidase and catalase in rice seed during germination. *Plant Physiol*. **52**: 274-237.

Riley, G.J.P.(1981). Effect of high temperature on germination of maize (Zea mays L.). *Planta* **151** : 68-74

Sairam, R.K., Srivastava, G.C. and Saxena, D.C. (2000). Increased antioxidant activity under elevated

temperature: a mechanism of heat stress tolerance in wheat genotypes. *Biol. Plant*. **43** : 245-251.

Savin, R. and Nicolas, M.E.(1996). Effect of short period of drought and high temperature on grain growth and starch accumulation of two malting barley cultivars. *Aust. J. Plant Physiol*. **23** : 201-210.

Singh, Mahak, Lallu, and Dixit, R.K. (2003). Studies on the high temperature tolerance in Indian mustard. National Seminar on stress management in oilseed for attaining self-reliance in vegetable oils, held at Directorate of Oilseed Research, Hyderabad.

Volaire, F., Thomas, H., Bertagne, N., Bourgeois, E., Gautier, M.F. and Lelievre (1998). Survival and recovery of perennial forage grasses under prolonged Mediterranean drought : water status, solute accumulation, abscisic acid concentration and accumulation of dehydrin transcript in bases of immature leaves. *New Phytol*. **140** : 451-460.

Yemm, E.W. and Willis, A.J. (1954). The estimation of carbohydrate in plant extracts by anthron. *Biochem. J.* **57**:508-514.

Zheng, Y.Z. and Han, Y.H. (1997). Effect of high temperature on drought stress on the activities of SOD and POD of intact leaves in two soybean cultivars. *Soybean Genet. News Letter* **24**: 39.