Research Journal of Agriculture and Biological Sciences, 3(4): 245-251, 2007


# Physiological Studies on Evaluation of Sunflower (*Helianthus annus* L.) Genotypes for High Temperature Stress

R. Amutha, S. Muthulaksmi, W. Baby Rani, K. Indira and P. Mareeswari

Regional Research Station, Aruppukottai - 626 107,
Tamil Nadu Agricultural University, Tamil Nadu, India

**Abstract:** An experiment was conducted at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu during 2005 to find out the response of Sunflower genotypes to high temperature stress. Twenty two sunflower genotypes were subjected to laboratory screening for high temperature stress through temperature induction response technique. The extent of thermotolerance was tested by assessing growth, TDMP, membrane damage, TTC reduction and proline content. The study revealed that induction response technique was highly advantageous in minimizing the adverse effects of high temperature stress. Gradual exposure of the sprouted seeds of sunflower genotypes to sub lethal stress before exposed to lethal stress caused significant enhancement in growth, physiological and biochemical characteristic of the seedlings.

**Key words:** *Temperature stress, Genotypes, Induction Response, Tolerance*

## INTRODUCTION

Ninety per cent of the world's sunflower production is confined to the tropical and semi-arid tropical regions, which are characterized by high temperature and low or erratic rainfall. High temperature of 38°C to 40°C causes reduction in seed yield, oil and protein content. In general, growing degree days (GDD) for sunflower ranges from 1042 to 1300 with a base temperature of 10°C. In India, sunflower is cultivated in two m ha. Though the average yield recorded in many countries is around 1,110 to 1500 kg $ha^{-1}$, the average yield obtained in India is around 550 kg $ha^{-1}$. The increase in total sunflower production over the years has been mainly due to increase in area from 0.1 m ha in 1980 to almost 2 m ha in 1999. However, the increase in productivity over these years is found to be insignificant [7].

Sunflower is predominantly grown as a rainfed crop. Although, this crop shows wide adaptability, the yield is destabilized by both abiotic and biotic stresses. Drought and high temperature are the two important abiotic constraints, which affect both vegetative and reproductive growths adversely. The coincidence of heat shock under water-limited condition is common in tropical and subtropical areas. Therefore, improvement for high temperature tolerance in sunflower is vital to stabilize the yield in these regions. With this idea in view this experiment was planned to find out the response of sunflower genotypes to high temperature stress.

## MATERIALS AND METHODS

An experiment was conducted at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu during 2005 to find out the response of Sunflower genotypes to high temperature stress. Seeds of 22 genotypes of sunflower, collected from the Department of Oilseeds, Tamil Nadu Agricultural University, Coimbatore, were used for the study. The imbibed seeds of sunflower were germinated on moist filter paper in Petri dishes at 30°C and 60 per cent relative humidity (RH) in a seed germinator for two days. On the third day, the induction response was given to the sprouted / germinated seeds. Protocols for temperature induction response developed by Mamatha Reddy [11] were followed for the study.

The sprouted seeds (two days old) of 22 sunflower genotypes were subjected to different induction temperature treatments at 35°C, 40°C and 45°C for four hours each or a gradual temperature induction, for which, temperature was increased from 35 to 45°C at the rate of 5°C per hour and it was maintained at 45°C for two hours. Later, the lethal temperature of 53°C was given. After the lethal temperature treatment, seedlings were allowed to recover by transferring them to 30°C for 72 hours. At the end of the recovery period, seedling height, TDMP, membrane integrity, TTC reduction and proline content were determined. For leaf membrane integrity, the measurement of leakage of solutes was done by absorption at ultraviolet wavelength of 273 nm [10]. Triphenyl tetrazolium chloride (TTC) reduction method

**Corresponding Author:** R. Amutha, Regional Research Station, Aruppukottai - 626 107, Tamil Nadu Agricultural University, Tamil Nadu, India

was done by the procedure of Palta *et al*. [12]. Proline accumulation in the leaf was estimated by the method of Bates *et al*. [2].

## RESULTS AND DISCUSSIONS

The temperature stress treatments viz., non induction (direct exposure to 53°C-1h) and induction (35°C-1h, 40°C–1h, 45°C–2h and 53°C-1h), imposed to 22 sunflower genotypes, resulted in significant changes in physiological characteristics of the seedlings. Direct exposure of the seedlings to lethal (non induced) temperature of 53°C for one hour caused an overall reduction of 26 per cent in seedling height (Table 1). This effect was attributed to poor signal perception and transduction and to the inability to express the stress responsive genes under lethal temperature stress [1]. The growth of the seedlings was, however, recovered when seedlings were initially subjected to induced (sub lethal) temperature with the subsequent exposure to non induced (lethal) temperature. The mean percentage recovery was, therefore, recorded to be 14.

**Table 1:** Effect of induction and non induction temperature treatments of sprouted seeds on height (cm) of sunflower genotypes (7 days old).

| | | Height (cm) | | | |
|---|---|---|---|---|---|
| Sl.No | Genotypes | Absolute control | Induced | Non induced | Mean |
| 1. | REC 435 | 7.88 | 5.87 (25.50) | 4.75 (39.78) | 6.17 |
| 2. | TNHAP 19 | 9.54 | 8.91 (6.55) | 7.42 (22.18) | 8.62 |
| 3. | R. Sel. Moster | 6.50 | 4.88 (24.94) | 4.26 (34.41) | 5.21 |
| 4. | ARM 242 | 7.96 | 6.57(17.52) | 4.03(49.37) | 6.19 |
| 5. | REC 428 | 7.60 | 6.42(15.53) | 5.16(32.10) | 6.39 |
| 6. | SANS 03 | 8.36 | 7.80(6.70) | 6.44(22.92) | 7.53 |
| 7. | DRSF 110R | 9.71 | 8.53(12.10) | 7.52(22.51) | 8.59 |
| 8. | ARSS 01 | 10.65 | 9.70(8.92) | 8.82(17.14) | 9.72 |
| 9 | TNHAP 3 | 12.30 | 10.00 (18.69) | 7.91(35.70) | 10.07 |
| 10. | Morden | 9.47 | 8.86(6.44) | 7.87(16.94) | 8.73 |
| 11 | CO 4 | 13.76 | 13.00(5.52) | 12.35(10.24) | 13.04 |
| 12 | TNAUSUF 214 | 11.24 | 10.11(10.09) | 9.08(19.21) | 10.14 |
| 13. | TNHSF 239 | 14.31 | 12.09(15.51) | 10.77(24.71) | 12.39 |
| 14. | EC 68415 | 13.60 | 10.31(24.16) | 8.18(39.83) | 10.70 |
| 15. | PS 1065 | 8.39 | 7.40(11.80) | 6.37(24.09) | 7.38 |
| 16. | PS 1012 | 11.29 | 9.80(13.15) | 8.50(24.71) | 9.60 |
| 17. | REC 441 | 10.52 | 8.75(16.87) | 7.88(25.14) | 9.05 |
| 18. | TNHAP 59 | 13.40 | 11.40(14.93) | 10.84(19.11) | 11.88 |
| 19. | R.Sel.Spec. | 12.26 | 9.50(22.55) | 7.92(35.44) | 9.89 |
| 20. | REC 443 | 9.54 | 7.67(19.55) | 6.65(30.29) | 8.10 |
| 21. | R.sel. Sartov | 13.21 | 10.63(19.53) | 8.85(33.00) | 10.90 |
| 22 | PS 1034 | 14.89 | 13.41(9.90) | 11.84(20.45) | 13.38 |
| | Mean | 10.74 | 9.16 | 7.88 | |

| | SEd | CD (0.05) |
|---|---|---|
| Genotypes | 0.24 | 0.48 |
| Treatments | 0.09 | 0.18 |
| Genotypes x Treatments | 0.41 | 0.83 |

**Table 2:** Effect of induction and non induction temperature treatments of sprouted seeds on TDMP (mg seedling$^{-1}$) of sunflower genotypes (7 days old).

| | | TDMP (mg seedling$^{-1}$) | | | |
|---|---|---|---|---|---|
| Sl.No | Genotypes | Absolute control | Induced | Non induced | Mean |
| 1. | REC 435 | 52.15 | 41.42(20.55) | 35.45(31.01) | 43.00 |
| 2. | TNHAP 19 | 64.38 | 48.78(24.23) | 47.60(26.07) | 53.59 |
| 3. | R. Sel. Moster | 58.39 | 45.12(22.72) | 35.94(38.44) | 46.48 |
| 4. | ARM 242 | 61.74 | 45.63(26.06) | 32.58(47.21) | 46.65 |
| 5. | REC 428 | 54.74 | 42.58(22.20) | 31.82(41.85) | 42.83 |
| 6. | SANS 03 | 59.34 | 51.44(13.31) | 42.67(28.08) | 51.15 |
| 7. | DRSF 110R | 62.90 | 52.35(16.76) | 43.84(30.30) | 52.03 |
| 8. | ARSS 01 | 60.52 | 53.26(12.00) | 42.61(29.60) | 52.13 |
| 9. | TNHAP 3 | 62.24 | 50.06(19.55) | 42.72(31.34) | 51.66 |
| 10. | Morden | 61.4 | 53.99(12.06) | 49.99(18.59) | 55.13 |
| 11. | CO 4 | 63.47 | 55.26(12.94) | 53.15(18.26) | 57.29 |
| 12. | TNAUSUF 214 | 63.69 | 56.16(11.83) | 46.63(26.79) | 55.49 |
| 13. | TNHSF 239 | 59.73 | 49.56(17.02) | 42.81(28.33) | 50.70 |
| 14. | EC 68415 | 59.70 | 43.12(26.15) | 33.77(43.43) | 45.53 |
| 15. | PS 1065 | 61.30 | 53.43(12.84) | 42.23(31.11) | 52.32 |
| 16. | PS 1012 | 60.00 | 54.13(16.16) | 42.47(29.21) | 53.72 |
| 17. | REC 441 | 65.27 | 49.12(24.75) | 43.80(32.89) | 53.73 |
| 18. | TNHAP 59 | 61.12 | 51.16(16.28) | 48.19(21.14) | 53.49 |
| 19. | R. Sel. Spec. | 56.88 | 45.38(20.21) | 38.18(29.36) | 47.48 |
| 20. | REC 443 | 58.30 | 47.60(18.36) | 38.64(32.87) | 48.18 |
| 21. | R. Sel. Sartov | 60.18 | 46.89(22.08) | 38.6(35.74) | 48.58 |
| 22. | PS 1034 | 64.20 | 52.95(17.53) | 46.12(28.16) | 54.42 |
| | Mean | 60.52 | 49.51 | 41.82 | |
| | | | SEd | | CD (0.05) |
| Genotypes | | | 1.28 | | 2.55 |
| Treatments | | | 0.47 | | 0.94 |
| Genotypes x Treatments | | | 2.21 | | 4.41 |

Among the 22 genotypes, CO 4, Morden, TNAUSUF 214 and TNHSF 239 were able to maintain higher growth even under non induced lethal temperature stress condition and, therefore, grouped as tolerant genotypes to temperature stress. The other genotypes viz., ARM 242, EC 68415, REC 439 and R.Sel.Spec. recorded poor growth, were grouped as susceptible genotypes. The response of tolerant as well as susceptible genotypes to induction temperature stress was very obvious in terms of seedling growth. The susceptible genotypes responded better than tolerant genotypes, particularly ARM 242, in terms of high recovery percentage of 63. The better improvement in seedling growth under induction treatment was well explained that the enhanced recovery of growth in induced seedling was attributed to stress response gene expression, resulting in higher accumulation of stress proteins [13].

Lethal temperature stress caused a greater reduction in TDMP (Table 2) of the sunflower genotypes. The most susceptible genotypes (ARM 242

**Table 3:** Effect of induction and non induction temperature treatments of sprouted seeds on membrane damage (%) of sunflower genotypes (7 days old).

| | | % damage | | | |
|---|---|---|---|---|---|
| Sl.No | Genotypes | Absolute control | Induced | Non induced | Mean |
| 1. | REC 435 | 12.1 | 37.2(207) | 65.5(441) | 38.26 |
| 2. | TNHAP 19 | 12.2 | 28.2(131) | 50.4(330) | 30.26 |
| 3. | R. Sel. Moster | 13.3 | 44.5(173) | 68.4(414) | 42.06 |
| 4. | ARM 242 | 12.4 | 35.5(186) | 75.00(504) | 40.96 |
| 5. | REC 428 | 14.00 | 41.1(170) | 69.2(394) | 41.43 |
| 6. | SANS 03 | 12.4 | 36.4(193) | 58.4(370) | 35.73 |
| 7. | DRSF 110R | 14.3 | 33.5(134) | 59.7(317) | 35.83 |
| 8. | ARSS 01 | 13.3 | 31.5(136) | 59.6(348) | 34.20 |
| 9. | TNHAP 3 | 13.5 | 29.7(107) | 65.00(381) | 36.60 |
| 10. | Morden | 11.2 | 25.5(127) | 45.6(307) | 29.33 |
| 11. | CO 4 | 12.4 | 26.5(113) | 40.00(222) | 26.30 |
| 12. | TNAUSUF 214 | 10.0 | 27.1(160) | 45.6(336) | 27.70 |
| 13. | TNHSF 239 | 12.4 | 28.2(127) | 53.3(329) | 31.30 |
| 14. | EC 68415 | 11.4 | 31.6(177) | 70.2(515) | 37.73 |
| 15. | PS 1065 | 12.3 | 32.5(164) | 52.2(324) | 32.33 |
| 16. | PS 1012 | 12.5 | 30.6(197) | 50.00(324) | 31.03 |
| 17. | REC 441 | 12.4 | 38.2(208) | 62.2(401) | 37.60 |
| 18. | TNHAP 59 | 13.4 | 36.2(170) | 57.4(328) | 35.66 |
| 19. | R. Sel. Spec. | 10.4 | 35.4(240) | 62.2(498) | 36.00 |
| 20. | REC 443 | 12.4 | 37.1(199) | 69.4(459) | 39.63 |
| 21. | R. Sel. Sartov | 12.3 | 41.2(234) | 65.2(430) | 39.56 |
| 22. | PS 1034 | 11.4 | 30.1(164) | 52.6(361) | 31.36 |
| | Mean | 12.38 | 33.53 | 58.95 | |

| | SEd | CD (0.05) |
|---|---|---|
| Genotypes | 0.667 | 1.333 |
| Treatments | 0.246 | 0.492 |
| Genotypes x Treatments | 1.156 | 2.309 |

and EC 68415) exhibited more than 40 per cent reduction over absolute control, whereas, the tolerant genotypes (CO 4 and Morden) recorded only 20 per cent reduction in seedling dry matter over control. The induction treatment resulted in better accumulation of dry matter and susceptible genotypes showed better response to induction temperature than the tolerant ones.

Membrane disruption is the first symptom of heat stress and membrane integrity (Table 3) is needed for thermotolerance [3]. High temperature stress causes damage to the organelle membranes, such as, nuclear membrane, endoplasmic reticulum, mitochondrial membranes and chloroplast membranes[5,6]. In the present study, lethal temperature caused more than 60 per cent damage to the cellular membrane. The induction temperature treatment was effective in minimizing the damage to 34 per cent and, therefore, the recovery percentage was estimated to be more than fifty. Increase in electrolyte leakage and loss of chlorophyll usually were attributed by membrane damage [14]. While observing the

**Table 4:** Effect of induction and non induction temperature treatments of sprouted seeds on TTC reduction (OD at 485nm) of sunflower genotypes (7 days old).

| | | OD at value (485 nm) | | | |
|---|---|---|---|---|---|
| Sl.No | Genotypes | Absolute control | Induced | Non induced | Mean |
| 1. | REC 435 | 0.624 | 0.501(19.71) | 0.388(37.82) | 0.504 |
| 2. | TNHAP 19 | 0.650 | 0.554(14.77) | 0.492(24.30) | 0.565 |
| 3. | R. Sel. Moster | 0.646 | 0.558(13.62) | 0.354(42.10) | 0.526 |
| 4. | ARM 242 | 0.634 | 0.498(21.45) | 0.354(44.16) | 0.495 |
| 5. | REC 428 | 0.622 | 0.483(22.34) | 0.382(38.59) | 0.496 |
| 6. | SANS 03 | 0.648 | 0.543(16.20) | 0.450(30.55) | 0.547 |
| 7. | DRSF 110R | 0.617 | 0.519(15.88) | 0.443(28.20) | 0.526 |
| 8. | ARSS 01 | 0.626 | 0.514(17.89) | 0.440(29.71) | 0.526 |
| 9. | TNHAP 3 | 0.684 | 0.583(14.76) | 0.493(27.92) | 0.587 |
| 10. | Morden | 0.651 | 0.589(9.52) | 0.584(10.29) | 0.608 |
| 11. | CO 4 | 0.722 | 0.633(12.33) | 0.631(12.60) | 0.662 |
| 12. | TNAUSUF 214 | 0.688 | 0.593(13.80) | 0.557(19.04) | 0.613 |
| 13. | TNHSF 239 | 0.651 | 0.589(13.54) | 0.584(15.71) | 0.608 |
| 14. | EC 68415 | 0.681 | 0.526(22.76) | 0.362(48.84) | 0.523 |
| 15. | PS 1065 | 0.682 | 0.572(16.13) | 0.473(30.62) | 0.576 |
| 16. | PS 1012 | 0.693 | 0.586(15.44) | 0.523(24.53) | 0.601 |
| 17. | REC 441 | 0.656 | 0.523(20.27) | 0.369(42.25) | 0.516 |
| 18. | TNHAP 59 | 0.621 | 0.538(13.37) | 0.479(22.87) | 0.546 |
| 19. | R. Sel. Spec. | 0.661 | 0.515(22.09) | 0.375(43.30) | 0.517 |
| 20. | REC 443 | 0.653 | 0.527(19.29) | 0.425(34.91) | 0.535 |
| 21. | R. Sel. Sartov | 0.623 | 0.462(25.84) | 0.366(41.25) | 0.484 |
| 22. | PS 1034 | 0.682 | 0.597(12.46) | 0.513(24.78) | 0.597 |
| | Mean | 0.655 | 0.545 | 0.456 | |

| | SEd | CD (0.05) |
|---|---|---|
| Genotypes | 0.014 | 0.029 |
| Treatments | 0.005 | 0.010 |
| Genotypes x Treatments | 0.025 | 0.050 |

response of the genotypes to induction temperature among the 22 genotypes, the susceptible ARM 242, showed higher response in terms of membrane stability.

The reduction of TTC (Triphenyl Tetrazolium Chloride), (Table 4) a reflection of mitochondrial functionality, indicates the cell viability under various stress levels. The decreased cell viability upon lethal heat treatment is attributed to the uncoupling of the inner mitochondrial membrane and inactivation of expenses of respiratory pathway. TTC acts as a substitute for oxygen as the final acceptor of electron from cytochrome a1 to a3 (cytochrome oxidase) of the mitochondrial electron transport chain [9]. Assessment of cell viability based on TTC reduction reveals both the structural and functional integrity of the respiratory apparatus [15]. The TTC reduction technique is well suited for assessing thermotolerance, because the two aspects of cell damage (membrane and enzyme stability) from heat stress can be evaluated at once. The results of the present study revealed that lethal temperature caused an adverse effect

**Table 5:** Effect of induction and non induction temperature treatments of sprouted seeds on proline content (µg $g^{-1}$ fr. wt.) of sunflower genotypes (7 days old).

| | | Proline content (µg $g^{-1}$ fr.wt.) | | | |
|---|---|---|---|---|---|
| Sl.No | Genotypes | Absolute ontrol | Induced | Non induced | Mean |
| 1. | REC 435 | 95.60 | 201.34(110) | 185.31(93) | 160.75 |
| 2. | TNHAP 19 | 85.87 | 215.26(150) | 176.29(105) | 159.14 |
| 3. | R. Sel. Moster | 93.33 | 217.28(132) | 178.26(90) | 162.95 |
| 4. | ARM 242 | 97.91 | 197.830(102) | 178.94(82) | 158.22 |
| 5. | REC 428 | 91.36 | 190(119) | 182.48(80) | 154.49 |
| 6. | SANS 03 | 88.67 | 218.38(146) | 178.89(101) | 161.97 |
| 7. | DRSF 110R | 76.59 | 253.95(231) | 157.81(106) | 162.78 |
| 8. | ARSS 01 | 90.27 | 227.48(151) | 186.91(107) | 168.22 |
| 9. | TNHAP 3 | 99.48 | 232.14(133) | 192.79(93) | 174.80 |
| 10. | Morden | 92.96 | 269.06(145) | 198.00(113) | 186.68 |
| 11. | CO 4 | 94.27 | 235.62(149) | 201.62(113) | 177.17 |
| 12. | TNAUSUF 214 | 92.05 | 238.24(158) | 185.84(101) | 172.04 |
| 13. | TNHSF 239 | 91.95 | 223.61(126) | 182.12(98) | 168.22 |
| 14. | EC 68415 | 74.13 | 185.61(150) | 132.00(78.06) | 130.58 |
| 15. | PS 1065 | 85.86 | 219.07(155) | 178.30(107) | 161.08 |
| 16. | PS 1012 | 93.98 | 236.57(151) | 193.78(106) | 174.77 |
| 17. | REC 441 | 97.49 | 216.78(122) | 181.02(85) | 165.09 |
| 18. | TNHAP 59 | 90.06 | 211.86(130) | 178.74(98) | 160.22 |
| 19. | R. Sel. Spec. | 98.64 | 216.67(119) | 181.28(83) | 165.53 |
| 20. | REC 443 | 92.03 | 174.7(89.92) | 163.28(77.42) | 143.36 |
| 21. | R. Sel. Sartov | 94.96 | 199.18(109) | 182.63(92) | 158.92 |
| 22. | PS 1034 | 96.08 | 234.32(141) | 192.98(100) | 179.09 |
| | Mean | 91.52 | 218.82 | 180.42 | |

| | SEd | CD (0.05) |
|---|---|---|
| Genotypes | 4.38 | 8.76 |
| Treatments | 1.62 | 3.24 |
| Genotypes x Treatments | 7.60 | 15.17 |

on TTC reduction and caused 30 per cent decline. Induction temperature, however, could modify this effect by improving the TTC reduction with the percentage recovery of about 20. Induced seedlings of sunflower exhibited high cell viability compared to non induced, as reflected by the high per cent reduction of TTC induced seedlings [13]. Similar results in bean, potato, soybean and tomato [4]. It was suggested that the induced seedlings exhibited better adaptation to the subsequent lethal stress, as reflected by the higher recovery in growth, high protein synthesizing capacity and maintenance of high cell viability.

Proline, (Table 5) an amino acid, imparts resistance to the plants to various kinds of stresses through its property of osmolyte. Under stress conditions, the concentration of proline increases up to 100 times the normal level, which makes upto 80 per cent of the total amino acid pool. In the literatures, the functions of the proline are well explained which include stabilization of proteins [16], scavenging of hydroxy radicals, regulation of cytosolic pH [16] and regulation of NAD / NADH ratio [1]. The per cent increase in proline content was 6, 25 and 125 in *Vigna radiata* seedlings subjected to heat shock at 40°C, 50°C and 60°C respectively. The present results also revealed a similar effect that proline content was increased by 100 per cent when subjecting the seedlings to lethal temperature. This content was increased further by 150 per cent when seedlings were subjected to induction (sub-lethal) temperature.

**Conclusion:** The study revealed that induction response technique was highly advantageous in minimizing the adverse effects of high temperature stress. Gradual exposure of the sprouted seeds of sunflower genotypes to sub lethal stress before exposed to lethal stress caused significant enhancement in growth, physiological and biochemical characteristic of the seedlings.

## REFERENCES


1. Alia and P.P. Saradhi, 1993. Biochem, Biophy. Res. Commun., 193: 54-58.
2. Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water stress studies. Plant Soil, 39: 205-207.
3. Blum, A. and A. Ebercon, 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci., 21: 43-47.
4. Chen, H.H., Z.Y. Shen and P.H. Li, 1982. Adaptability of crop plants to high temperature. Crop Sci., 22: 719-725.
5. Ciamporova, M. and J. Mistrik, 1993. The ultra structural response of root cells to stressful conditions. Environ. Exp. Bot., 33: 11-26.
6. Collins, G., X.L. Nie and M. Saltveit, 1995. Heat shock proteins and chilling sensitivity of mungbean hypocotyls. J. Exp. Bot., 46: 795-802.
7. Damodaran, T. and D.M. Hidge, 1999. Oilseed Situation: a statistical compendium, ICAR, DOR, Rajendranagar - 500 030.
8. Das, D.R. and S. Mukherji, 1994. Changes in sugar, starch and proline contents of *Vigna radiata* (L.) seedlings after heat shock on seeds during early imbibitions. Indian J. Plant Physiol., 37: 59-60.
9. Kalina, M. and J.M. Parmer, 1986. The reduction of tetrazolium salts by mitochondria. Histochemic., 14: 366-374.
10. Leopold, A.C., M.E. Musgrave and K.M. Williams, 1981. Solute leakage resulting from leaf desiccation. Plant. Physiol., 68: 1222-1225.
11. Mamatha Reddy, M., 2000. Development of stress tolerant lines in sunflower (*Helianthus annuus* L.) by adapting TIR techniques. M.Sc. (Ag.) Thesis, University of Agricultural Sciences, Bangalore.
12. Palta, J.P., H.H. Chen and P.H. Li, 1981. Relationship between heat and frost resistance of several potato species. Effect of cold adaptation on heat resistance. Bot. Gaz., 142: 311-315.
13. Ramesh Kumar, B.M., 2000. Identification of thermo tolerant competitive parental lines to develop stress resistant hybrids in sunflower. Ph.D. Thesis, University of Agricultural Sciences, Bangalore.
14. Simon, E.W., 1974. Phospholipids and plant membrane permeability. New Phytol., 73: 377-420.
15. Towell, L.E. and Mazur, 1974. Studies on the reduction of 2,3,5-tri phenyl tetrazolium chloride as a viability assay for plant tissue culture. Can. J. Bot., 53: 1097-1102
16. Venekamp, J.H., 1989. Physiology plant., 76: 112-113.