Thermo tolerance in *Brassica*: refined rapid screening method to identify thermo tolerant genotypes in *Brassica*

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Abstract

Brassica being a crop of arid and semi arid region, its sowing depends upon rain. Due to early rains, many times the farmers have to sow the crop early in the season on the conserved moisture. High temperature at that time causes seedling mortality. This necessitates thermotolerant genotypes. The present paper deals with simple, rapid and refined methodology suitable for screening exhaustive germplasm. Sowing is done in plastic trays containing soil. Each tray can accommodate 3-4 rows, and each row has 6-7 spots depending upon the dimensions of the tray. Four seeds are sown on each spot. Sowing is done in three trays as replicates. Seeds are allowed to germinate inside the seed germinator at optimum temperature and humidity. Five days old seedlings are exposed to threshold high temperature. Time taken to 50% seedling mortality is used as the criteria to assess thermotolerance. Longer the time taken to 50 % mortality, more a variety will be tolerant to high temperature. The main advantages of this method are:

I. The chances of error are the least, as the large numbers of genotypes being tested are in a single lot having similar microclimatic conditions.
II. Method is rapid as the thermotolerance of large number of genotypes can be tested in a few hours.
III. As the testing is done in natural soil, this method is similar to natural soil conditions rather than using petri plate/ fitter paper methods, which are purely laboratory methods.
IV. Time taken to seedling mortality is better testing tool than percent seedling mortality.
V. Verification of the above-proposed method was done by testing thermo tolerance of many species of *Brassica* and whose tolerance/susceptibility is already established.

Key words: *Brassica,* Thermotolerance, Screening Techniques, Heat stress.

Introduction

Brassica being a crop of arid and semi arid region, its sowing depends upon rains. In India, many a times, due to early rains, farmers sow the crop early in the season. Such early sown crop faces seedling mortality High temperature prevailing during sowing time causes seedling mortality to the tune of 100%. Therefore crop needs to be re sown many times before a final successful crop is taken.

Among the many approaches to combat this problem, selection of thermotolerant genotypes is the best approach, as it is one time effort and does not involve regular expenses of farmers. The present paper deals with developing simple, and laboratory method corresponding to field conditions for screening thermotolerant genotypes.

Materials and methods

Raising the seedlings: Sowing is done in trays having normal field soil. In the present experiment 3 trays were used. Each tray could accommodate 7 Kg. soil therefore, 21 Kg. soil was homogenized out side the tray with enough but measured amount of water to bring the soil to field capacity. In the present experiment 150ml water/Kg. soil was used. Equal quantity of soil was filled in each box. Each box was uniformly marked into 4 rows and 7 spots were marked in each row at uniform distance (Fig.1). Four seeds were sown at each spot. Therefore, each tray could accommodate 4 seeds each of 28 genotypes. Sowing was done in random fashion in 3 trays, Seedlings were allowed to grow at optimum temperature (25°C $\pm$ 1°C), relative humidity (70%) and optimum light conditions for five days. Then these seedlings were exposed to threshold high temperature (46 $\pm$ 1°C).

Trays were kept in automatic controlled seed germinator at 25°C, and 50% relative humidity. Five days' old seedlings were then exposed to high temperature (46 $\pm$ 1°C). Time taken to bending initiation (hypocotyl hook formation), 50% bending, 50% mortality, and 100% mortality was recorded. Longer the time needed for a variety/genotype to 50% mortality, more a variety/ genotype will be tolerant to high temperature compared to the one, which takes lesser time to reach that stage. Based upon this trait the genotypes were grouped into thermo tolerant, moderately thermo tolerant, and thermo susceptible genotypes. Efforts were done to screen some of the species/genotypes whose thermotolerance is already established.

Results and Discussion

Screening of thermotolerant genotypes can be achieved by screening thermotolerant genotypes either in field or laboratory conditions. The limitation of screening in field is that temperature cannot be controlled to threshold limit. Resulting thereby either all the seedlings are killed or none is killed. Moreover, microclimatic conditions of different seedlings may vary in field area.

In laboratory, the seedlings can be grown in Petri plates, in moistened blotting papers or boxes/trays containing soil. In petri plates there is very high relative humidity, which is against the natural field conditions where high temperature is accompanied by low relative humidity and water stress. None of the conditions is met in Petri plates.

Screening of genotypes in wrapping seeds in moistened blotting papers too have drawback, as the conditions are unnatural. Such seedlings are protected against high temperature and water stress. To overcome this difficulty a simple laboratory method has been suggested as mentioned in materials and methods that is comparable to natural field conditions.

Verification of proposed method by screening the established thermo tolerant and thermo susceptible species of *Brassica*: Six species of *Brassica* (Listed in Table1) were tested for their comparative thermotolerance at seedling stage by using the proposed method of screening.

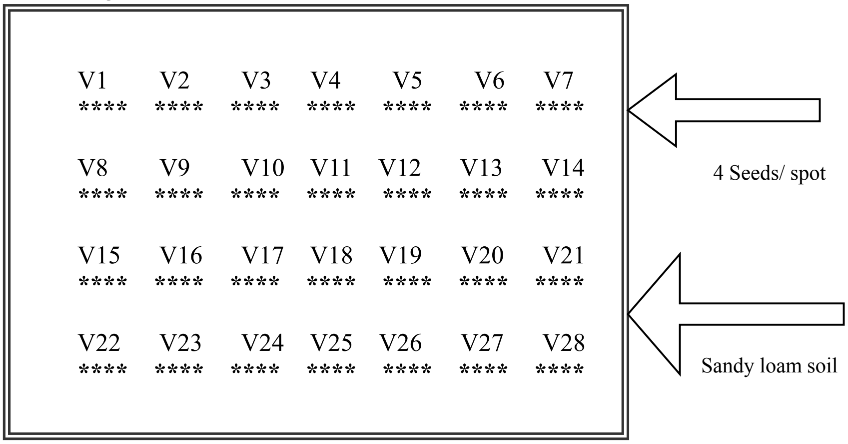

Fig. 1. Sowing pattern of *Brassica* seedlings. V= variety/genotype, * indicates placement of seeds in tray.

A wide variability was found in time taken to 50% mortality in 3 genotypes of B.juncea group. Genotype RH-8813 took only 2 hrs. to attain 50% seedling mortality, whereas RH-9707 took 3hrs.20 min. and RH-8816, took extremely long time i.e. 5 hrs. to reach 50% seedling mortality stage. It shows that RH-8816 is relatively a thermo tolerant genotype of *B.juncea*. Much lesser differences were observed in time taken to 50% mortality in 3 different genotypes of *B. campestris*. Genotypes TH-9807, TH-68 & Sangam took 5 hrs, 5hrs.-15 min. and 5hrs.-30 min. respectively. Therefore variety Sangam is relatively more thermo tolerant than TH-68, and TH-9807.In *B. napus* genotypic differences were too small viz: GSH-1 & HNS-9605 took 3 hrs.-15 min. to attain 50% seedling mortality, whereas, GSL-1 took 3hr 30 min. to achieve this stage. Similarly in *B. carinata*, Kiran & HC-9605 took 5 hrs-45 min. Whereas WF took-5hr. 30 min. to attain 50% mortality. *B. tournifortii* took 5hrs. and *B. alba* took only 3hr.-45 min. to 50% mortality.

Data on mean time taken by different genotypes of each species to achieve 50% & 100% seedling mortality was compared. Among these species, *B.carinata* took maximum time to achieve 50% seedling mortality and therefore has been ranked the most thermo tolerant species followed by *B. campestris* and *B. tournifortii,* which were almost at par with each other. These 3 species were much more tolerant than *B.alba, B. juncea,B..napus.* The most susceptible to high temperature is *B.napus*.

Thermotolerance and drought tolerance in *B. carinata* is evident from the proposed screening method is well established in literature (AICORPO, 2000, Chhabra & Promila, 1998, Chhabra, 2000). It is recommended for rain fed condition and is believed to be rough & tough *Brassica* species (Rapaport & Ferereas, 1983).). *B.carinata* is also relatively tolerant to water stress (Uprety & Tomar, 1995), salinity stress (Chhabra, 1999), high temperature stress (Chhabra, 2000) & frost (Chhabra, 1998).

B.campestris var Toria is also proven thermo-tolerant through indirect observations. The recommended sowing time to *B.campestris* var. Toria in India is September because *Brassica* species of *Brassica* can tolerate high temperature of September when the temperature is high whereas, there is seedling mortality in *Brassica juncea if* sown in this month. That is the reason; the sowing of *Brassica juncea* is recommended in October when temperature lowers down.

Thermo-susceptibility of *B.napus* is supported by the fact that it is cultivated in areas where lands are irrigated & have high relative humidity. It is less suitable for water stress condition or dry condition. *B.juncea* though not tolerant to high temperature is most widely cultivated, because yield wise no other species can compete it for one or the other reason. Though B.juncea as a whole is not thermo-tolerant, there is range of thermo-tolerance in different genotypes of B.juncea.

To further confirm the method, a set 25 coded genotypes including thermotolerant genotypes RH-8814 and RH-8816 were screened for confirmation. Genotypes RH-8814 and RH-8816 took 6-20 and 5-30hrs. respectively to achieve 50% mortality compared to 3-30hrs. in thermo-susceptible genotype (RH-8113).This further support the proposed method.

On an overall, though this method seems to be only slight improvement over the existing methods, offers many advantages. Superiority of this method over the existing methods are:

1. The chances of error are the least, as the large numbers of genotypes are being tested are in a “single tray” having similar microclimatic conditions.
2. Method is rapid as the thermotolerance of large number of genotypes can be tested in a few hours.
3. As the trays contain natural soil, this method is similar to natural soil conditions rather than Petri plate/ moistened filter paper methods, which are purely laboratory techniques.
4. Time taken to seedling mortality is more reliable testing tool than percent seedling mortality.

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Table 1: Effect of high temperature (46±1°C) on time taken (hrs-min) to seedling mortality in different species of *Brassica*.

	B. juncea			*B. rapa*			*B. napus*			*B. carinata*			*B. tournifortii*	*B. alba*
	RH-8113	RH-9707	RH-8816	TH-9807	TH-68	Sangam	GSH-1	HNS-9605	GSL-1	Kiran	HC-9605	WF	Local	Local
50 % mortality	2-00	3-20	5-00	5-00	5-15	5-30	3-15	3-15	3-30	5-45	5-45	5-30	5-00	3-45
Mean		3-30			5-15			3-20			5-40		5-00	3-45
100%mortality	4-00	5-00	6-30	6-30	6-30	6-45	4-30	4-45	4-30	7-00	6-45	6-45	6-30	5-00
Mean		5-10			6-35			4-35			6-50		6-30	5-00