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IN VITRO REGENERATION OF SOME SELECTED MUSTARD GENOTYPES (*Brassica spp*)

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ABSTRACT

An experiment was conducted to find out *in vitro* performance of some mustard genotypes (Safal, Sampad and Tori-7). For germination, MS medium was used without any growth regulators. For callus induction, 2, 4-D was used @ 1.5 mg/L concentration and for regeneration, Indole Acetic Acid (IAA) as well as Benzyl Amino Purine (BAP) were used @ 0.5 mg/L and 1.0 mg/L, respectively. The Plant Growth Regulators were used as a single dose combination to ascertain only the genotypic differences. In genotypic sense, Tori-7 and Safal showed better performances compared to Sampad (92.73% and 94% callus induction for Tori-7 and Safal, respectively). On the other hand, genotypes Tori-7 and Sampad ensured better regeneration (72% for Tori-7 and 65% for Sampad) than that of Safal (50%). Therefore; Tori-7 may be termed as the best genotype in terms of both callus induction and regeneration. No statistically significant differences were found for the genotypes regarding days required for callus induction but in case of days required for regeneration, the genotypes varied significantly. Both the genotypes Safal (38.98 days) and Sampad (41.79 days) are early regenerating than Tori-7 (45.21 days). All of the genotypes under study have shown their suitability in tissue culture studies with varying abilities.

Key words: *In vitro*, regeneration, mustard genotypes.

Introduction

Next to Soybean and Palm, *Brassica* has occupied a prominent place in world's agrarian economy as oil seeds, vegetables, feed and fodder, biofuels, condiments and green manure (Gupta and Pratap, 2007). About 13.2% of the World's edible oil supply comes from this crop (Downey and Robbelen, 1989). In Bangladesh it remains top in the list in respect of area and production, among the oil crops grown in the country (Mondol and Wahhab, 2001). It covers 61.2% of the total oilseeds acreage of the country and 52.6% of the total production (BBS, 1999). The average yield of *Brassica* oilseed in Bangladesh is around 740 kg ha^{-1}(Chowdhury and Zulfikar, 2001; Mondal and Wahhab, 2001). Bangladesh is still facing acute shortage in edible oils (BBS, 2003). Therefore, achieving higher yield in a lower acreage of land is the demand of this moment. With a view to doing this, there is an exigency to make an improvement in both agronomic and genetic approaches of mustard. Due to high degree of segregation upon cross-pollination and unavailability of suitable germplam, conventional breeding alone was not successful enough in *Brassica*. Moreover, abiotic and biotic stress infestation has made the problem compounded. Under these circumstances, *in vitro* regeneration and transformations have prospects to fulfill breeding needs (Khan *et al.,* 2010). *Brassica spp.* has become an object of extensive tissue culture studies and breeding. To date organogenesis has been achieved in a variety of explants such as stem sections (Pua *et al*., 1993) and hypocotyls (Phogat *et al*., 2000). Genetic modification of crop is rapidly becoming the technique of choice for the production of new agricultural varieties. An efficient regeneration protocol for different genotypes of *Brassica* is needed to be established for its use in transformation experiments in order to produce transgenic of required characteristics. The present study is an attempt to develop and standardize an efficient regeneration system for different genotypes of *Brassica.*

Materials and Methods

Three different genotypes of *Brassica* (Safal, Sampad, Tori-7) were obtained from local farmers of Jamalpur District for this study. For surface sterilization, *Brassica* seeds were washed with tap water and

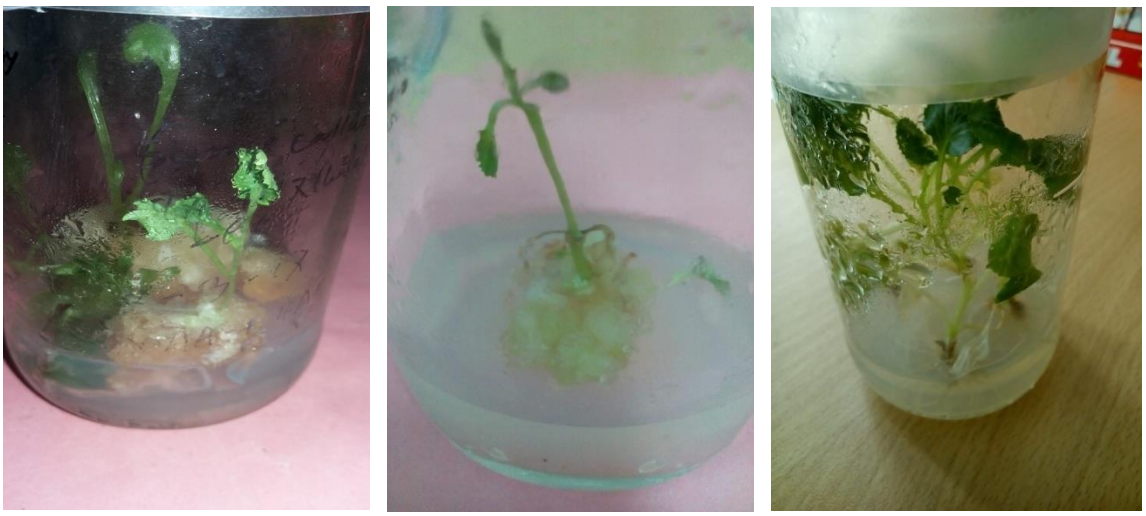
dipped in 70% ethanol for 30 seconds. Then the seeds were treated with Sodium hypochlorite solution (1% active chlorine) for 20-25 minutes followed by rinsing 3-4 times with sterilized distilled water**.** After sterilization, seeds were cultured for germination on half strength MS medium (Murashige & Skoog, 1962). The laboratory experiment was conducted at Molecular Breeding Laboratory of Patuakhali Science and Technology University during the period from February 2019 to April 2019. Hypocotyl portion was selected as explants for *in vitro* callus induction and Shoot proliferation aseptically under suitable growth condition. MS medium supplemented with Sucrose (30.0 g/l), different concentrations of auxins (2,4-D and Indole Acetic Acid) and cytokinins (Benzyl amino purine). The pH was adjusted within a range of 5.6 to 5.8 and agar was added to the solution @ 8 g/l (0.8 % w/v). The medium was poured on autoclaved bottles and sterilized by autoclaving (at 121 ºC and 15 psi) for 15 min was used to induce callus and shoots from all the 3 genotypes. The growth regulators were added @1.5 mg/L 2, 4-D, 0.5 mg/L IAA and 1.0 mg/L BAP. Explants were taken from 5-6 days old seedlings. The hypocotyls portions were inoculated on MS medium supplemented with different concentrations of plant growth regulators. Cultures were incubated at 25ºC ± 2 under 16 h light/8 h dark conditions and observed regularly for shoot formation and callus initiation. Data were recorded on daily basis and parameters were days required for callus initiation, shoot induction rate (%), callus induction rate (%) and days required for regeneration. The Analysis of Variance was performed by Fischer's statistics and mean values were compared by Duncan's Multiple Range Test.

Results and Discussion

Significant variation was observed for *in vitro* performance among genotypes for both callus induction and regeneration (Fig. 1). For callus induction, the highest percentage of callus was obtained for Safal (94%) followed by Tori-7 (92.73) and Sampad (83.80%). There was no significant statistical variation between Safal and Tori-7 in this regard. There was also no significant variation for days required for calli induction. The study also revealed that Tori-7 exhibited 72% regeneration which is the highest followed by Sampad (65%) and Safal (50%). On the other hand, Genotype Safal showed earliest regeneration (38.98 days) and Tori-7 showed late regeneration (45.21 days). The results denote more suitability of genotype Tori-7 for efficient regeneration achievement compared to other two genotypes (Table 1).

A B C

A. Callus Induction in Genotype Safal, B. Callus Induction in Genotype Sampad, C. Callus Induction in Genotype Tori-7



D E F

D. Regeneration in Genotype Safal, E. Regeneration in Genotype Sampad and F. Regeneration in Genotype Tori-7

Fig. 1: *In vitro* performance of some mustard genotypes

Table 1: *In vitro* Performance of different mustard genotype

Genotypes	Total number of explants	Total number of Callus Induction	Callus Induction %	Days for Calli Induction	Regeneration %	Days Required for Regeneration
Safal	15	14.10	94a	8.10a	50b	38.98b
Sampad	15	12.57	83.80b	6.25a	65a	41.79b
Tori-7	15	13.91	92.73a	6.90a	72a	45.21a

Genotype Sampad performed better than Safal considering regeneration ability. Similar results have been found by Ghosal *et al* 2008, Guo *et al* 2005 and Dubey and Gupta 2014.

Conclusion

The present study has been successful to identify efficient regeneration protocol and difference between suitable tissue culture responsive genotypes which could be exploited as potential source and bridge material for further transformation and tissue culture studies.

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