An Efficient *in vitro* Regeneration System for Tori (*Brassica campestris*)-7

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**Abstract** - An efficient *in vitro* regeneration system for Tori (*Brassica campestris*) -7 was developed from calli of hypocotyls and cotyledonary leaves. The optimum medium for callus induction was found with 0.5 mg/l 2, 4-dichloro-phenoxyacetic acid (2,4-D). The best shooting medium contained 3.0 mg/l 6, benzyl amino purine (BAP), 0.1 mg/l naphtalene acetic acid (NAA), and 5.0 mg/l AgNO₃. Maximum number of shoots were produced when 0.5 mg/l kinetin was used, whereas the combined effect of 2.0 mg/l BAP, 0.1 mg/l NAA, and 5.0 mg/l AgNO₃ regenerated calli the most. The effect of AgNO₃ was found for callogenesis at 0.5 mg/l and for regeneration at 5.0 mg/l. *In vitro* regenerated shoots of Tori-7 developed roots on medium with 1.0 mg/l indole 3-butyrlic acid (IBA). The developed efficient *in vitro* regeneration protocol can be used as a baseline for Agrobacterium-mediated genetic transformation of the studied plants.

**Keywords** : agrobacterium, *in vitro* regeneration, and tori-7.

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An Efficient in vitro Regeneration System for Tori (Brassica campestris)-7
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Abstract: An efficient in vitro regeneration system for Tori (Brassica campestris)-7 was developed from calli of hypocotyls and cotyledonary leaves. The optimum medium for callus induction was found with 0.5 mg/l 2, 4- dichlorophenoxyacetic acid (2,4-D). The best shooting medium contained 3.0 mg/l 6, benzyl amino purine (BAP), 0.1 mg/l naphtalene acetic acid (NAA), and 5.0 mg/l AgNO₃. Maximum number of shoots were produced when 0.5 mg/l kinethin was used, whereas the combined effect of 2.0 mg/l BAP, 0.1 mg/l NAA, and 5.0 mg/l AgNO₃ regenerated calli the most. The effect of AgNO₃ was found for callogenesis at 0.5 mg/l and for regeneration at 5.0 mg/l. in vitro regenerated shoots of Tori-7 developed roots on medium with 1.0 mg/l Indole 3-butyric acid (IBA). The developed efficient in vitro regeneration protocol can be used as a baseline for Agrobacterium-mediated genetic transformation of the studied plants.

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I. INTRODUCTION

Mustard (including rape) is one of the most important crops and accounts 3.08% of the total production of major crops in Bangladesh. The production of oil seed in 1994-95 was 307,602 metric tons in the country, from which 179,524 metric tons of oil was produced that can supply only 27% of the daily per head oil need. Hence, we need to increase annual Brassica production. Improvement of Brassica production rate can be achieved through in vitro tissue culture of mustard. Tissue culture or in vitro micropropagation technique has been applied for Brassicas for a long time observed by Ali et al. (2007) and John et al. (1991). The combination of 2,4-D, BAP, NAA and AgNO₃ was found to be the best medium for callus initiation and growth for Brassica napus by Ali et al. (2007). The use of AgNO₃ was reported by Khan et al. (2003) in Brassica napus and Ali et al. (2007) for plant regeneration. However, the best condition for Bangladeshi Brassica local variety (Tori-7) regeneration system is not known. The present study was designed to screen out the varietal response of Tori-7 regarding its response to specific cultural conditions in comparison with and finally, to establish an efficient genotype-independent in vitro cultural system for the initiation and development of embryogenic calli with an ultimate goal of plant regeneration that can be used in future as a baseline for Agrobacterium-mediated genetic transformation for quality improvement.

II. MATERIALS AND METHODS

Tori-7 (Brassica campestris) seeds were collected from Bangladesh Agricultural Research Institute (BARI), Hathazari Substation, Chittagong. The explants collected from in vitro grown seedlings used for the experiments were: hypocotyl and cotyledons with petiole. Hypocotyl segments of 0.5-1.0 cm and cotyledonary leaves with petioles were used for the experiments. MS (Murashige and Skoog, 1962) medium was prepared with 3% (w/v) sucrose and solidified with 0.4% (w/v) agar. Only half strength MS medium was used for seed germination whereas, MS media supplemented with different PGRs (Plant Growth Hormones) such as 2, 4-D, BAP and additives such as proline and casein hydrolysate (CH) were used for callus induction. Shoots were developed by using media with BAP and the effect of hormones and additives were checked. Initiation of roots were tried on media supplemented with NAA and IBA. The plantlets with sufficient rooting system were taken out of the culture vessels and the roots were washed under tap water. The in vitro grown rooted plants were then transferred into small pots. Hardening was carried out by periodical exposure of the plants to natural environment.

III. RESULTS AND DISCUSSIONS

Cotyledons and hypocotyls, both formed large calli on MS medium supplemented with 0.5 mg l⁻¹ 2, 4-D. The increasing concentration of 2, 4-D greatly reduced the percentage of callus formation as well as size of the callus (Fig.1), though this effective concentration of 2,4-D does not agree with many previous reports such as Cardoza and Stewart (2003), who found the best callus induction using 1.0 mg l⁻¹ 2, 4-D. Quain and Zhang (2004) and Khan et al. (2002) reported best callus induction medium using 2,4-D at 1.5 mg l⁻¹ and 2.0 mg l⁻¹, respectively. These differences with the optimal concentration of 2, 4-D of current investigation can be assumed as a result of varietal differences and variations in the exo- and endogenous environments. The use of BAP on callogenesis was found to increase the size and frequency of callus formation. Withdrawal of BAP from the medium reduced

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the percentage of hypocotyl callus formation even when 2, 4-D concentration was increased to 1.0 mg l⁻¹. The explants became brown that did not produce any significant callus (Fig. 2). On the other hand, withdrawal of AgNO₃ from the medium caused the explants become brown and dead (data not shown). The use of AgNO₃ was described to improve the growth of the callus by Noman et al. (2008) that supports the result. About 12% of the hypocotyl calli and 1-2% of the cotyledonary explants produced roots within 20 days of culture on medium containing 0.5 mg l⁻¹ IBA and 0.5 mg l⁻¹ IAA (Fig. 3). Hypocotyls produced callus of embryogenic nature on proline containing medium though cotyledons responded less in proline and CH contacting media (Fig. 3). The increasing BAP reduced shooting (Fig.4). When NAA at 0.1 mg l⁻¹ was used in combination with BAP, the percentage of shoot formation raised to 80% from 47.8% for hypocotyl explants. George and Rao (1980) observed maximum regeneration from cotyledon explants in Brassica juncea on medium supplemented with BAP and NAA rather than BAP only, which supports the present findings. About 20% cotyledonary calli started shooting within 10-12 days. The medium without AgNO₃ but with NAA gave shoots at a percentage of 65-80% (data not shown). Presence of CH in media reduced shooting frequency.

Spontaneous root generation occurs sometimes on MS medium with hormonal supplements for the induction of shoots (Fig.5). Tori-7 responded only on media containing IBA (Fig.5). This finding again suggests the genotypic and environmental difference may cause the variety to respond.

On the basis of observation and results taken, Tori-7 was better in terms of producing more shoots but the subsequent growth and establishment was difficult that is supported by Dunwell (1981), Dietert et al. (1982) and Glimelius (1984) who concluded the difficulties of regeneration of Brassica campestris than other Brassicas.
IV. Conclusion

The present study has described an efficient in vitro regeneration system for a local Brassicas variety namely Tori-7. The optimum medium for callus induction was found with 0.5 mg/l 2, 4-D, whereas the best shooting medium was 3.0 mg/l BAP, 0.1 mg/l NAA, and 5.0 mg/l AgNO₃. Maximum number of shoots were produced when 0.5 mg/l kinetin was used, whereas the combined effect of 2.0 mg/l BAP, 0.1 mg/l NAA, and 5.0 mg/l AgNO₃ regenerated calli the most. In vitro regenerated shoots of Tori-7 developed roots on medium with 1.0 mg/l indole 3-butyric acid (IBA). The developed efficient in vitro regeneration protocol will be supportive for increasing the local productivity.

REFERENCES Références Referencias


Fig. 5 : Different stages of regeneration; spontaneous root formation on shooting medium unattached to shoots (A), shoot elongation from Tori-7 hypocotyls on medium containing kinetin (B) and formation of rooting system of Tori-7 on IBA containing medium (C)
