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Original Research Article

Callus Induction and Shoot Proliferation from Seedling Explants of Different Mustard Genotypes

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A B S T R A C T

Due to the growing world population and increasing industrialization, the demand for edible oil and biofuels is increasing, thus cultivation of oilseed crops has gained great importance. The present work concentrates on the study of different concentrations of Plant growth regulators for callus induction and shoots proliferation of three genotype of *Brassica* namely MM (*Brassica napus*), GM (*Brassica juncea*) and YM (*Sinapis hirta*). Result indicated that hypocotyl portion found best for callus induction in MS+0.5mg/l 2, 4-D +0.5mg/l NAA (94%). Maximum shoot proliferation percentage was recorded with MM- MS+0.5mg/l BAP+0.5mg/l Kinetin (100%) as well as with GM- MS+1.0mg/l BAP (100%) but It was noted that genotype MM showed shoot proliferation relatively at higher rates. It was also recorded that MM (100%) for *in vitro* regeneration and GM (86.6%) for *in vivo* seed germination gave best results and cultivar YM regeneration capability among the three cultivars was very slow in both case (*in vitro* and *in vivo*). These results can be used in further evaluation of different genotype of *Brassica* in transformation experiments in order to produce transgenic of required characteristics and in clonal propagation as well.

Keywords

Regeneration, Shoot proliferation, Callus induction, Plant Growth Regulators.

Introduction

The oilseed *Brassica* species cultivation has increased tremendously during the last decade and by now it is the second largest contributor to the world supply of vegetable oil (Zhou, 2001). Many *Brassica* species are also important vegetable crops, e.g., cole crops (*Brassica oleracea*). Several species, e.g., *Brassica carinata*, *Camelina sativa*, *Crambe abyssinica*, *Eruca vesicaria*, have potential as new edible oil/protein crops, biodiesel fuel crops, or platforms for bioproducts or molecular farming (Gugel and Falk 2006, Warwick and Gugel 2003, Warwick et al., 2006, 2007).

Due to the growing world population and increasing industrialization, the demand for edible oil and biofuels is increasing; thus cultivation of oilseed crops has gained great importance (Indrajit et al., 2008).

Conventional breeding approaches can be done to improve the new trait within the species. But Conventional breeding programmes alone were not successful enough in *Brassica* due to high degree of segregation (the separation of allelic genes that occurs typically during meiosis) upon cross-pollination and unavailability of

suitable wild germplasm Enrichment of genetic variability through mutation, somaclonal variation, and protoplast fusion contributed only a little in the production of disease and pest resistant plants to overcome incompatibility barriers as well as plants with better agronomic characters in *Brassica spp.* In this regard, *in vitro* regeneration and transformation have prospects to fulfill breeding needs (Khan *et al.*, 2010). *Brassica napus* 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 entitled "Callus Induction and Shoot Proliferation from Seedling Explants of Different Mustard Genotype." is an attempt to develop and standardize an efficient regeneration system for different genotypes of *Brassica.*

Materials and Methods

Three different genotypes of *Brassica* (MM, GM and YM) were obtained from Naveen Seeds Centre Raipur, and brought to the Devleela Biotechs, Anand Vihar Colony, V.I.P. Road, Raipur (C.G.) for further 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) and MS medium.

Callus induction and Shoot Proliferation

Hypocotyl and Nodal 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, α-naphthalene acetic acid) and cytokinins (Benzyl amino purine and Kinetin). The pH was adjusted within a range of 5.6 to 5.8 and agar was added to the solution at a rate of 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.

Explants were taken from 3-5 days old seedlings. The hypocotyls and Nodal portion were discarded and were inoculated on MS medium supplemented with different concentrations of plant growth regulators. Cultures were incubated at $25^{o}C \pm 2$ under 16 h light/8 h dark conditions and observed regularly for shoot formation and callus initiation. Rooted shoots were transferred to the soil in pots and acclimatized to the greenhouse in 25 to 35^{0}C temperature and 65 to 80% Relative humidity. Data were recorded on daily basis and parameters were germination, shoot induction and callus induction rate (%).

Results and Discussion

The present experiment entitled "Callus Induction and Shoot Proliferation from Seedling Explants of Mustard Genotype". Result indicated that Sarita-333 (MM) (100% *in vitro* germination rate) was reported as best variety among others for *in vitro* regeneration.

Surface Sterilization - Combination of 70% Alcohol + 0.1% $HgCl_2$ for 5 minutes was

recorded as best surface sterilization with lowest rate of contamination. Germination rate in Green house - GM (86.6% *in vitro*) for *in vivo* seed germination gave best results. Result showed on Table No 4.1.

In vitro Shoot proliferation - MM- MS+0.5mg/l BAP+0.5mg/l Kinetin (100%) as well as with GM- MS+1.0mg/l BAP (100%) but It was noted that genotype MM showed shoot proliferation relatively at higher rates. The shoot proliferation was obtained from inoculation of Nodal portion of all variety was showed on Table No. 4.2, 4.3 and 4.4. Callus induction - Hypocotyl portion found best for callus induction in MS+0.5mg/l 2, 4-D +0.5mg/l NAA (94%). The callus induction was obtained from inoculation of Hypocotyls part of all variety was showed on Table No. 4.5, 4.6 and 4.7.

In present investigation different concentration and combination of growth regulator were used to define an efficient shoot proliferation and callus induction medium. 2, 4-D and BAP were alone and combination with NAA and Kinetin increased the percentage of callus induction respectively according to variety of different explants. Selection of a suitable variety should depend upon the climatic and soil requirement of plant. As we were carrying out the experiments in Chhattisgarh, it was necessary to select a suitable variety of *Brasscia*, which best studied to the local climate and soil.

Table. 4.1 Seed Germination rate in Green house

S. No.	Variety	No of Seeds	Germination rate (days/cm)				
			0	**1**	**2**	**3**	**4**
1	MM	30	0	G	G	1.2	2.7
2	GM	30	0	G	G	2.0	3.5
3	YM	30	0	No	No	G	1.2

Table. 4.2 Shoot Proliferation on MM variety

S. No.	Medium	% of Shooting	Shoot height	No of Shoots
1	MS+0.5mg/l BAP	100	4.2	09
2	MS+1.0mg/l BAP	100	3.9	25
3	**MS+0.5mg/l BAP+0.5mg/l Kinetin**	**100**	**8.0**	**21**
4	MS+1.0mg/l BAP+0.5mg/l Kinetin	100	6.0	13

Table. 4.3 Shoot Proliferation on GM variety

S. No.	Medium	% of Shooting	Shoot height	No of Shoots
1	MS+0.5mg/l BAP	100	3.8	07
2	**MS+1.0mg/l BAP**	**100**	**3.3**	**25**
3	MS+0.5mg/l BAP+0.5mg/l Kinetin	100	6.5	11
4	MS+1.0mg/l BAP+0.5mg/l Kinetin	100	7.2	18

Table. 4.4 Shoot Proliferation on YM variety

S. No.	Medium	% of Shooting	Shoot height	No of Shoots
1	MS+0.5mg/l BAP	25	3.2	3
2	MS+1.0mg/l BAP	22	3.1	2
3	**MS+0.5mg/l BAP+0.5mg/l Kinetin**	**35**	**6.5**	**8**
4	MS+1.0mg/l BAP+0.5mg/l Kinetin	20	8.0	4

Table. 4.5 Callus Induction (Hypocotyl) on MM variety

S. No.	Medium	% of Callusing	Weight of callus (Dry/gm)
1	MS+0.5mg/l 2, 4-D	75	0.248
2	MS+1.0mg/l 2, 4-D	86	0.211
3	**MS+0.5mg/l 2, 4-D +0.5mg/l NAA**	**94**	**0.354**
4	MS+1.0mg/l 2, 4-D +0.5mg/l NAA	82	0.175

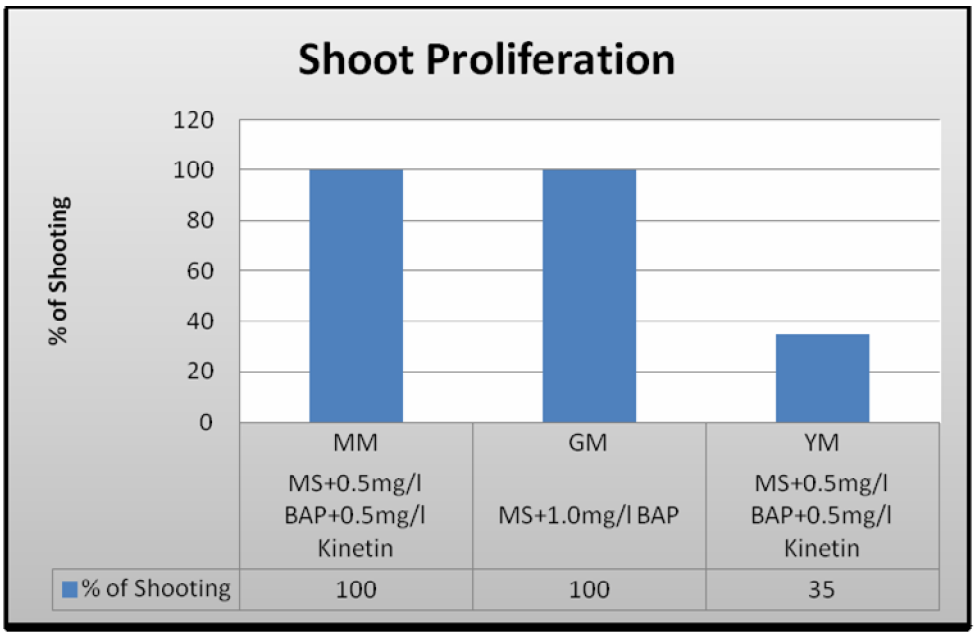
Table. 4.6 Callus Induction (Hypocotyl) on GM variety

S. No.	Medium	% of Callusing	Weight of callus (Dry/gm)
1	MS+0.5mg/l 2, 4-D	79	0.145
2	**MS+1.0mg/l 2, 4-D**	**91**	**0.526**
3	MS+0.5mg/l 2, 4-D +0.5mg/l NAA	82	0.265
4	MS+1.0mg/l 2, 4-D +0.5mg/l NAA	64	0.169

Table. 4.7 Callus Induction (Hypocotyl) on YM variety

S. No.	Medium	% of Callusing	Weight of callus (Dry/gm)
1	MS+0.5mg/l 2, 4-D	23	0.089
2	MS+1.0mg/l 2, 4-D	26	0.124
3	**MS+0.5mg/l 2, 4-D +0.5mg/l NAA**	**31**	**0.179**
4	MS+1.0mg/l 2, 4-D +0.5mg/l NAA	37	0.156

Fig. 1 Shoot proliferation of different variety from Nodal Portion



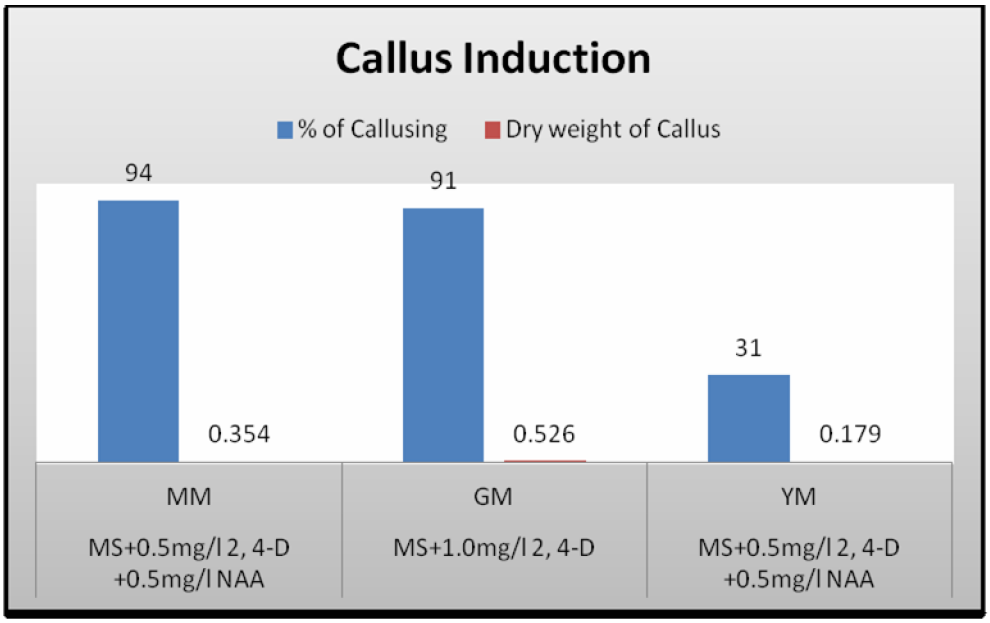
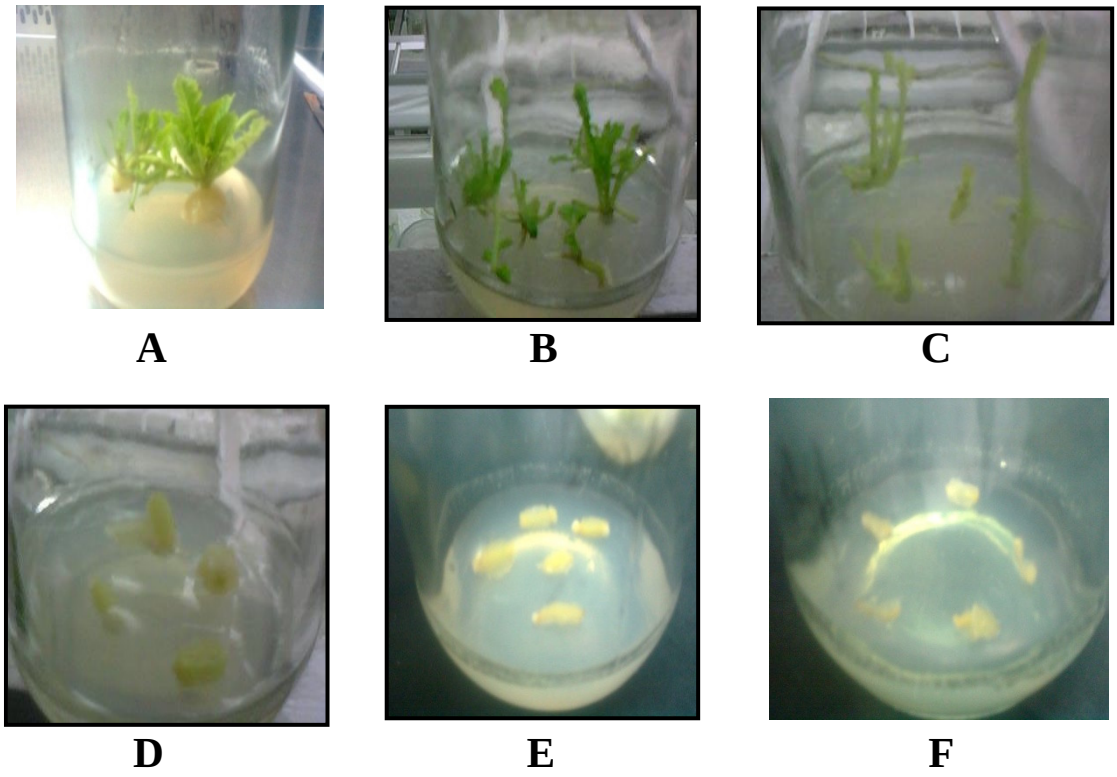


Fig.3 Callus indication and Shoot proliferation on different concentration of PGR.



(A)Shoot proliferation from Nodal portion of MM - MS+0.5mg/l BAP+0.5mg/l Kinetin. **(B)GM -** MS+1.0mg/l BAP. **(C)YM -** MS+0.5mg/l BAP+0.5mg/l Kinetin. **(D)Callus induction from Hypocotyl of MM -** MS+0.5mg/l 2, 4-D +0.5mg/l NAA. **(E)GM -** MS+1.0mg/l 2, 4-D. **(F)YM -** MS+0.5mg/l 2, 4-D +0.5mg/l NAA.

Hypocotyl portion gave best for callus induction in MS+0.5mg/l 2, 4-D +0.5mg/l NAA (94%) (Table No 5) was best whereas Al-Naggar et al (2010) reported that the best callus initiation and maintenance was achieved by the supplementation of 0.5 mg l-1 2, 4-D for hypocotyls explants and 1.5 mg l-1 2, 4-D when the cotyledon explants were used as source of callus initiation. Bano Raisa et al., 2010, were selected two phytohormones, auxins (Naphthalene acetic acid and Indole acetic acid) and cytokinins (Benzylaminopurine and Kinetin) with concentrations were used to develop an efficient regeneration protocol for 3 genotypes of *Brassica juncea* (UCD-635, RL-18 and NIFA RAYE).

Maximum callus production (65.55) was observed on MS medium containing with BAP 2.0 mgL-1/NAA 0.2 mg L-1. Maximum shooting (22.31) was observed BAP 3.0 mg L-1/NAA 0.3 mg L-1 and KIN 3.0 mg L-1/IAA 0.3 mg L-1. Regeneration efficiency was found maximum (7.13) with BAP 3.0 mg L-1/NAA 0.3 mg L-1 but present study showed that for shoot proliferation nodal portion *in vitro* in MS+0.5mg/l BAP+0.5mg/l Kinetin (100%) (Table No 2) was indicated optimum regeneration. Yellow Mustard (*Brassica Hirta)* has very slow growth rate as compare to others (MM and GM). It was also noted that MM (100%) for *in vitro* regeneration and GM (86.6%) (Table No 1) for *in vivo* seed

germination (Greenhouse) gave best results.

References

Al-Naggar A., Shabana R., Rady M., Ghanem S., Saker M., Reda A., Matter M. and Eid S., 2010. In *vitro* callus initiation and regeneration in some canola varieties. Int. J. Acad. Res., 2: 6–12.

Bano R., Khan M.H., Khan R.S., Rashid H. and Swati Z.A. 2010. Development of an efficient regeneration protocol for three genotypes of *Brassica juncea*. Pak. J. Bot., 42: 963–969.

Gugel R.K., Falk K.C. 2006. Agronomic and seed quality evaluation of *Camelina sativa* in western Canada. Can J Plant Sci 86:1047–1058.

Indrajit D., Prasenjit S. and Sampa D. 2008. Efficient *Agrobacterium*-mediated genetic transformation of oilseed mustard [*Brassica juncea* (L.) Czern.] using leaf piece explants. *In Vitro Cell. Dev. Biol. Plant*, 44: 401-411.

Khan M., Robin A., Nazim-Ud-Dowla M., Talukder S. and Hassan L. 2010. *In vitro* Regeneration Potentiality of *Brassica* Genotypes in Differential Growth Regulators ISSN 0258-7122 Bangladesh J. Agril. Res. 35(2): 189-199, June.

Murashige T. and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant*., 15: 473-379.

Phogat S.K., Burma P.K. and Pental D. 2000. High frequency regeneration of *Brassica napus* varieties and genetic transformation stocks containing fertility restorer genes for two cytoplasmic male sterility systems. J. Plant Biochemistry and Biotechnology 9: 73 - 79.

Pua E.C. and Chi G.L., 1993. De novo shoot morphogenesis and plant growth of mustard (*Brassica juncea*) *in vitro* in relation to ethylene. Physiol. Plant., 88: 467–474.

Szulc P. and Drozdowska L. 1997. The effect of kind of explant and growth regulators on plant regeneration of Polish winter oilseed genotypes. Rosliny Oleiste.

Warwick S.I., Gugel R. 2003. Genetic variation in the *Crambe abyssinica* – *C. hispanica* – *C. glabrata* complex. Genet Resour Crop Evol 50:291–305.

Warwick S.I., Gugel R., McDonald T. 2006. Genetic variation and agronomic potential of Ethiopian mustard (*Brassica carinata*) in western Canada. Genet Resour Crop Evol 53:297–312.

Warwick S.I., Gugel R.K., Gomez-Campo C. 2007. Genetic variation in the *Eruca vesicaria* (L.) Cav. Plant Genet Resour Charact Util 5:142–153.

Zhou W.J. 2001. Oilseed rape. In: Crop Cultivation. (Eds.): G.P. Zhang and W.J. Zhou. Zhejiang University Press, Hangzhou, China, 153-178.