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In vitro callus induction and plantlet regeneration is influenced by the maturity status of embryos of *Brassica rapa* varieties

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ABSTRACT

Brassica rapa (AA, 2n = 20) is a prime oilseed species in Bangladesh that can be improved via biotechnological approaches. Rescue of developing embryos after an interspecific hybridization, which otherwise usually tend to abort, is very important for improvement of this species, that require an efficient embryo culture protocol. The immature torpedo shaped embryos (451-700 µm) and mature walking-stick type (>700 µm) embryos of five B. rapa varieties namely, Safal, Agrani, BINA Sarisha-6, BARI Sarisha-6, BARI Sarisha-15 were cultured in basal MS media supplemented with 1 mgL^{-1} BAP, 0.5 mgL^{-1} NAA & 1 mgL^{-1} 2,4-D. The immature embryo culture followed indirect somatic embryogenesis process but the mature embryos followed direct organogenesis. The immature embryos induced callus within 11-15 days whereas the mature embryos regenerated plantlets through direct organogenesis within 8-11 days. The varieties Agrani, BARI Sarisha-6 and BINA Sarisha-6 induced the highest percentages of callus from immature embryos and the varieties Safal and BARI Sarisha-15 regenerated the highest percentage of plantlet from mature embryos. Safal and Agrani induced callus and regenerated plantlets comparatively earlier than other varieties. An increase in the concentration of BAP from 10 to 20 ppm in MS media + 5 ppm IBA decreased the size of the plantlets of all varieties except the Agrani. The data could be used in improving the existing embryo rescue protocols. The direct organogenesis process of mature embryos can potentially shorten the breeding cycles.

Key words: *Brassica rapa, embryo culture, callus, phytohormone, plantlet, 2'4-D and basal MS media*

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I. Introduction

Brassica species, commonly known as mustard and rapeseed is an important oilseed and vegetable crop which contributes around 12% of the world's total edible vegetable oil production. It is the major oil crop in Bangladesh contributing nearly 71.3% of the total oilseed production. *Brassica* spp. is also

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used as a food flavoring, for forage, as an emetic, and diuretic, as well as a topical treatment for inflammatory conditions such as arthritis and rheumatism etc. (Ahmad and Abdin, 2000; Malek et al., 2014; McVetty and Duncan, 2015; Rakow, 2004; Ram Manohar et al., 2009). Among the oil producing *Brassica* species, *B. rapa* (AA, 2n = 20) is the prime species in Bangladesh. Mustard and rapeseed (*Brassica* spp.) is cultivated in almost 0.6 million acres of land with the average yield of 371 kg/acre, producing a total of 222 thousand metric tons in Bangladesh (BBS, 2014). This average yield of *Brassica* in Bangladesh is, however, very low compared to the many mustard growing countries of the world (Ara et al., 2014; Mila et al., 2014).

In *Brassica*, inter-specific hybridization is one of the most important technique of crop improvement via transferring traits between species of commercial interest as the genetic background of *Brassica* offers the unique possibility of hybridization within its species (Seyis and Aydin, 2014). Interspecific hybridization is used to develop synthetic hexaploid and has been widely used for improving *Brassica* species (Choudhury et al., 1990; Inomata, 2012; Zou et al., 2011). For example, it was observed that the traits of economic importance, such as disease resistance, early maturity, could be transferred from *B. carinanta* and *B. rapa* via interspecific hybridization between these two species (Choudhury et al., 1990).

In an inter-specific cross the resultant embryo may be aborted because of parental mutual incompatibility or abnormal meiosis (Brown and Brown, 1996). This can be overcome by immature embryo culture, a process that can rescue inherently weak, immature or hybrid embryos to prevent degeneration (Ćosić et al., 2013; Hilgert-Delgado et al., 2015; Maheswaran and Williams, 1986). The successful application of this technique depends on the stage of the embryo being rescued and cultured *in vitro* (Finkelstein and Crouch, 1986). The requirements for culture of the embryos become less complex with the increasing maturity of the embryos (Quazi, 1988; Uma et al., 2011). A high possibility of damage during rescue and sensitivity to osmotic shock under *in vitro* culture are added difficulties in the culture of young embryos compared with older ones (Raghavan and Srivastava, 1982). Fully matured embryos were shown to regenerate directly into plantlets (Uma et al., 2011). It is thus established that along with possible other factors of *in vitro* culture system, the success of the rescue of inter-specific embryos, depends largely on embryo maturity, and which was not properly addressed for the *Brassica* varieties of Bangladesh. This current research was therefore, designed and materialized to optimize the *in vitro* culture system of immature and mature embryo of five selected *B. rapa* species.

II. Materials and Methods

Preparation of explants: Seeds of five *B. rapa* varieties *viz.* Safal, Agrani, BINA Sarisha-6, BARI Sarisha-6, BARI Sarisha-15 were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur and Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Seedlings were raised in the field of BINA and siliquas of different ages were collected. Embryos of different shapes *viz*., globular, heart (Figure 01a), torpedo (Figure 01b) and walking-stick type, as determined by the relative age, size and shape of embryos, were dissected using a stereoscopic dissecting microscope in a chamber under laminar flow. The torpedo shaped embryos were categorized as immature embryos (451-700 µm) and walking-stick type embryos were categorized as mature embryos (>700 µm).

Explant culture: Excised mature and immature embryos of different ages from the five varieties, with five replicates for each, were cultured directly in MS (Murashige and Skoog, 1962) medium supplemented with sucrose and with different concentrations of hormones *viz*., 1 mgL^{-1} 6-Benzylaminopurine (BAP), 0.5 mgL^{-1} 1-naphthaleneacetic acid (NAA) & 1 mgL^{-1} 2,4-Dichlorophenoxyacetic acid (2,4-D) for callus induction following a Completely Randomized Design (CRD). The explants were incubated under fluorescent light with controlled temperature (22±2°c), photoperiod (16 hrs) and relative humidity (75%). The cultures were observed regularly to record their callus formation and regeneration response. At every stage, the contaminated vials were immediately discarded from the stock. Almost after 14-15 days of inoculation, different morphological callus phenotypes were noticed. The immature embryos produced callus and the mature embryos produced plantlets directly.

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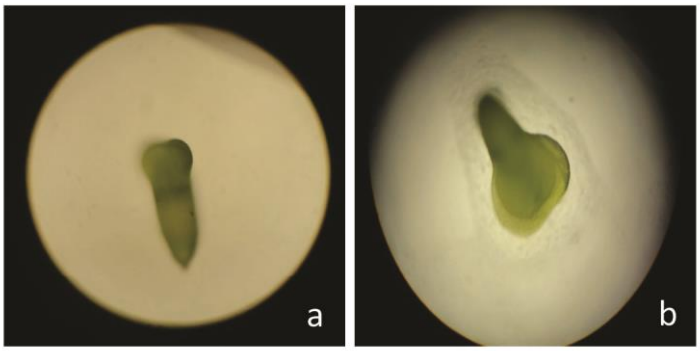

Figure 01. Images showing (a) torpedo (size range 451-700 µm) and (b) heart shaped (size range 81-450 µm) embryos of 22 days old siliqua of *Brassica rapa* variety 'Agrani'. Images were taken under dissecting microscope at 100x magnification.

Subculture of calli induced from immature embryos: The calli, induced from immature embryos, attained convenient size, usually after 15-16 days after inoculation of explants, were sub-cultured aseptically on freshly prepared sterilized medium containing the same hormonal supplements. The vials showing signs of contamination were discarded on regular basis and data were collected from the growing calli.

Proliferation of shoot of the plantlets produced from mature embryo: The plantlets, induced from mature embryos, of convenient size were transferred to shoot induction media (basal MS media supplemented with 4 ppm BAP to proliferate shoots. When these shoots grew about 3-4 cm in length, they were rescued aseptically from the cultured vials and were separated from each other and again sub-cultured individually on vials with freshly prepared shoot induction medium (MS). But this time MS medium was supplemented with two different hormonal combinations, one with 10 ppm BAP and 5 ppm Indole-3-butyric acid (IBA) and another with 20 ppm BAP and 5 ppm IBA to observe the effect of these hormonal combinations on the proliferation of plantlets. The vials containing shoots were incubated at 25±1°C under controlled environmental condition. The proliferated shoots were finally transferred to the rooting media (Basal MS media supplemented with 4 ppm IBA) to initiate root formation. Day to day observations was carried out to note the responses of shoot formation.

Collection and analysis of data: The varietal performances in various callus parameters such as percentages of callus initiation, days to callus initiation, size, colour and texture of callus etc. were recorded from the immature embryo culture. While both the varietal performance and the effects of two different hormonal conditions were observed via various plant regeneration parameters such as percentage of plant regeneration, days required to plant regeneration and size of the plantlets etc. were studied from the regenerated plantlets from mature embryo culture. The analysis of variance for different characters was performed and means were compared by the Duncan's Multiple Range Test (DMRT). The data were analyzed using the MSTATc.

III. Results

The regeneration potentiality of the torpedo (size range 451-700 µm) and heart shaped (size range 81-450 µm) embryos (representing the mature and immature embryos, respectively) of five *B. rapa* varieties, namely Safal, Agrani, BINA Sarisha-6, BARI Sarisha-6, BARI Sarisha-15 were tested using tissue culture methods. Immature embryos followed indirect somatic embryogenesis (produced callus) while mature embryos followed direct organogenesis (produced plantlets).

In vitro callus induction from immature embryo

The torpedo shaped immature (451-700 µm) embryos were cultured on MS media supplemented with 1 mgL^{-1} BAP, 0.5 mgL^{-1} NAA & 1 mgL^{-1} 2,4-D and the data on various callus parameters such as percentages of callus initiation, days to callus initiation, size, colour and texture of callus were recorded.

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Variability for different callus parameters

Significant varietal difference was observed for the parameters days required for callus initiation and size of callus while colour and texture of callus varied insignificantly between the varieties (Table 01).

Table 01. Analysis of variance (Mean square values) for days to callus initiation

Sources of Variation	Degrees of Freedom	Mean Sum of Square			
		Days required for callus initiation	Size of callus (cm^2)	Colour of callus	Texture of callus
Variety	4	14.16***	0.46***	2.24^{NS}	0.06^{NS}
Error	*20*	*0.82*	*0.6*	*0.7*	*0.3*

*** and ** indicate significant at 0.1 % and 0.5% level of probability, respectively and ^{NS} indicates non-significant

Percentages of callus induction

The varieties Agrani, BINA Sarisha-6 and BARI Sarisha-6 have shown the highest percentage (80%) of callus induction followed by that of BARI Sarisha-15 (60%). The lowest percentage of callus induction (40%) was recorded in Safal (Figure 2). The images of initiated calli are shown in Figure 07.

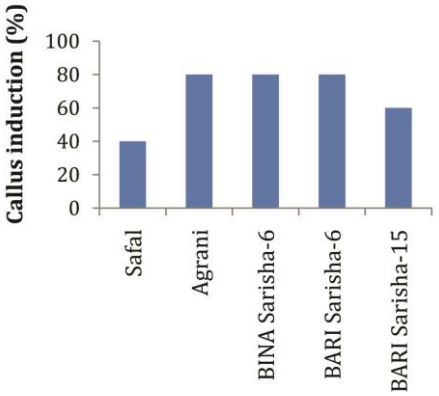

Figure 02. The percentage of callus induction in torpedo shaped immature embryo (451-700 µm) embryos of five different Brassica rapa varieties namely Safal, Agrani, BINA Sarisha-6, BARI Sarisha-6 and BARI Sarisha-15 when cultured on basal MS media supplemented with 1 mgL-1, 0.5mgL-1NAA and 1mgL-1 2,4-D. Data recorded on 20 days after inoculation.

Days required for callus initiation

Callus were initiated more quickly in the variety BARI Sarisha-6 (11.4 ± 0.4 days) followed by that of BINA Sarisha-6 (11.6 ± 0.24 days) compared to the other varieties (Figure 03). However, the days required for callus initiation did not differ significantly between these two varieties. For callus initiation, the highest number of days were required by the varieties Safal and Agrani (both 15 ± 0.45 days) followed by BARI Sarisha-15 (13.8±0.49 days). The differences for days required for callus induction in these three varieties were statistically significant with that of BARI Sarisha-6 and BINA Sarisha-6 ($P<0.01$) as shown in Figure 03.

Size of the callus

Size of the callus were measured with a scale of small (0.49 cm^2), medium (1.83 cm^2) and large (2.25 cm^2) and the variety BARI Sarisha-15 had the largest callus followed by the variety Safal. Variety Agrani produced the smallest calli among the all varieties when cultured on the same media with phytohormone 1 mgL^{-1} BAP,0.5 mgL^{-1} NAA and 1 mgL^{-1} 2,4-D as shown in Figure 03.

Colour and texture of the callus

The colour of the 20 days old calli was recorded visually using a scale of 1 to 5 grading 1 for whitish, 2 for creamish, 3 for yellowish, 4 for light green and 5 for green callus. All the varieties produced

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yellowish to green callus except the variety Agrani which produced creamish calli. The visual score were analyzed; it was observed that Agrani is significantly different than other four varieties as determined by DMRT test (Figure 04).

Similar trend of results were also observed for texture of callli which was measured based on a scale of 1 to 3 where 1 was graded for friable callus, 2 for loose and 3 for compact calli where Agrani once again show statistically significant different performance for texture of callus than four other varieties even though the calli produced by all the varieties ranged between loose to compact.

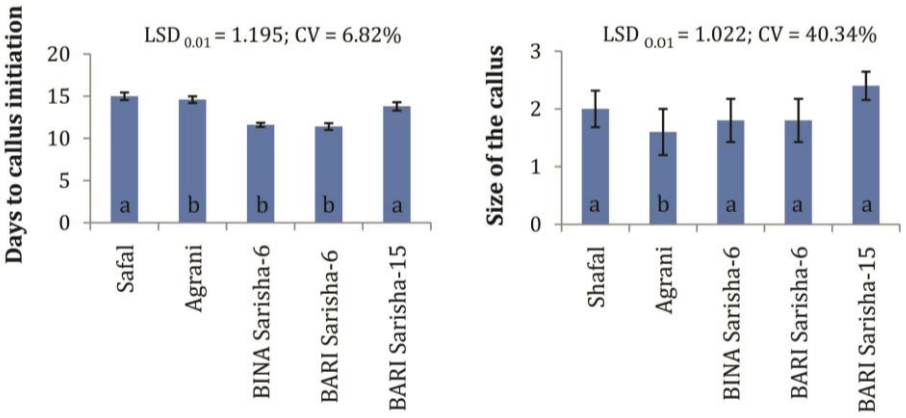

Figure 03. Bar graph showing the days required for callus initiation from the immature torpedo shaped embryo (22 days old) and the size of the callus (cm2) at 20 days after callus induction of five *Brassica rapa* varieties cultured on MS media supplemented with 1 mgL-1 BAP, 0.5 mgL-1 NAA & 1 mgL-1 2, 4-D. The size of callus was determined as small (0.49 cm2), medium (1.83 cm2) and large (2.25 cm2). Data presented as mean (n=5) ± SE and different letters on the bars indicate significant difference ($p < 0.05$) by DMRT analysis.

In vitro plantlet regeneration from mature embryo

Walking stick type mature embryos of more than 26 days old with a size range of >700 µm were cultured initially in MS media supplemented with 1 mgL^{-1} BAP, 0.5 mgL^{-1} NAA and 1 mgL^{-1} 2,4-D to observe the varietal performance for percent plantlet regeneration and days required to plantlet regeneration. The regenerated plantlets were then sub-cultured in two different media compositions to observe both varietal difference and effect of phytohormome combinations in the size of regenerated plantlets.

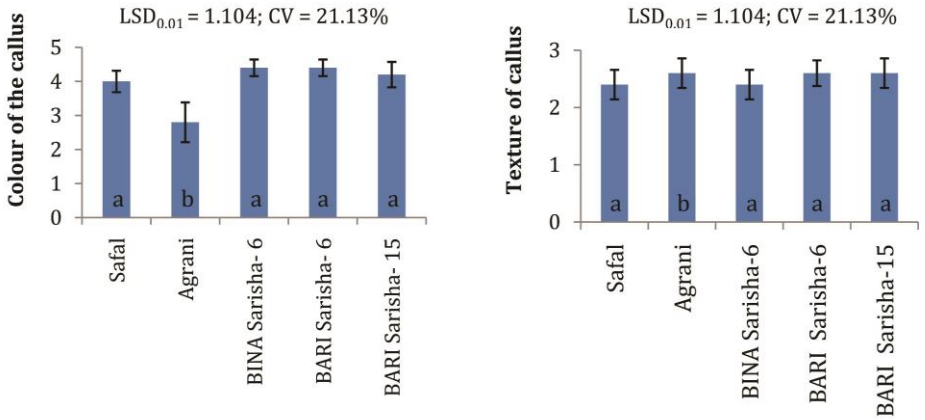

Figure 04. Bar graph showing the color and the texture of 20 days aged calli obtained from culturing immature torpedo shaped embryos of five different *Brassica rapa* varieties. The colour was recorded using a scale of 1 to 5 with 1 being scored for whitish, 2 for creamish, 3 for yellowish ,4 for light green and 5 for green callus. The texture of callli was measured based on a scale of 1 to 3 where 1 was graded for friable callus, 2 for loose and 3 for compact calli. Data presented as mean (n=5) with

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vertical bars indicating standard error of mean. LSD value indicates least significant differences between treatments. Different letters on the bar indicate significant difference by DMRT analysis.

Percent plant regeneration

The mature embryos regenerated plantlets directly following direct organogenesis. The five different varieties have shown differential response for plant regeneration from mature embryos. The variety Safal (40%) and BARI Sarisha-15 (60%) has shown less callus induction percentages from immature embryo culture (Figure 02). The same varieties showed the highest plant regeneration percentages (88.8% and 73.33%, respectively) from the mature embryo culture. The rest three varieties *viz*., Agrani, BINA Sarisha-6 and BARI Sarisha-6 produced more than 70% plant regeneration from mature embryo culture (Figure 05). The images of regeneration plantlets are shown in Figure 07.

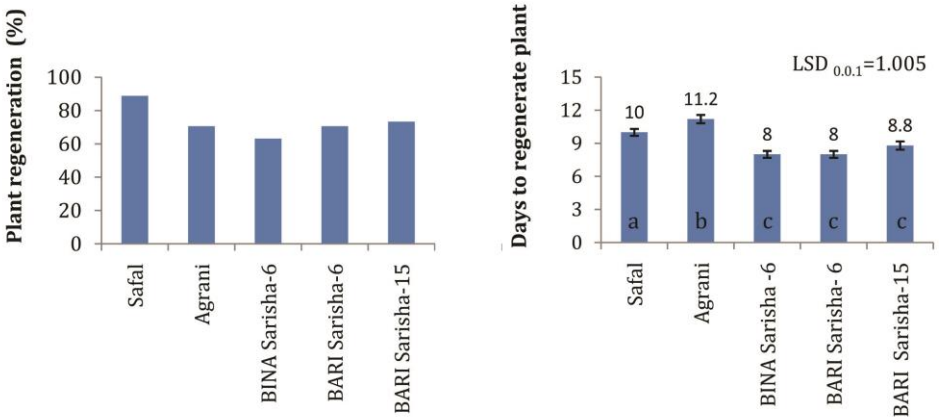

Figure 05. Bar graph showing the percentages of plantlet regeneration and days required for plantlet regeneration from mature walking-stick type (26 days old, >700 µm) embryos of five different *Brassica rapa* varieties namely, Safal, Agrani, BINA Sarisha-6, BARI Sarisha6 and BARI Sarisha-15 when cultured on basal MS media supplemented with 1 mgL^{-1} BAP, 0.5 mgL^{-1} NAA and 1 mgL^{-1} 2,4-D. Data recorded on 15 days after inoculation and presented as mean of 5 replicates. LSD indicates least significant differences and data presented as mean (n=5) ± SE (for days required to plantlet regeneration) and different letters over the bar indicate statistically significant difference as determined by DMRT analysis.

Days required to regenerate plantlets

Statistically significant varietal difference was observed for the days required to plantlet regeneration as determined by ANOVA (Mean sum of square value = 9.60, $p<0.01$). The varietal response for this trait showed somewhat different trend than that of percentage of plantlet regeneration. The varieties BARI Sarisha-6, BINA Sarisha-6 regenerated plantlets more quickly (8 days) followed by BARI Sarisha-15 (8.8 days) whereas the variety Agrani took maximum days to regenerate plantlets (11.2 days) followed by the variety days (10 days) (Figure 05).

Size of plantlets

When the regenerated plantlets attained a convenient size, the plantlets were subculture in freshly prepared media. But this time MS medium supplemented with two different hormonal combinations, one with 10 ppm BAP and 5 ppm IBA and another with 20 ppm BAP and 5 ppm IBA were used to observe the effect of these hormonal combinations on the proliferation of plantlets in terms of their size. Significant differences were observed between varieties, treatments and their interactions for the size of the sub-cultured plantlets (Table 02). In general, the increase in the concentration of BAP from 10 ppm to 20 ppm in the media composition did not shown an increase in the size of the plantlets. In fact, it decreased in the varieties BINA Sarisha-6, BARI Sarisha-6 and BARI Sarisha-15 and remained unchanged in variety Safal. Only in the variety Agrani an increase in the size of plantlets were observed (Figure 06). The varieties BINA Sarisha-6 and BARI Sarisha-15 had shown largest plantlets (2.8 cm) at 10 ppm BAP whereas the variety Safal produced smallest plantlets (1.2 cm) of all five varieties.

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Table 02. Mean square values for size of the plantlets at two different treatments

Sources of Variation	Degrees of freedom	Sum of square	Mean sum of square	F - ratio
Variety (V)	4	8.88	2.22 *	5.05
Treatment (B)	1	1.62	1.62 *	3.68
V × T	4	4.88	1.2 *	2.77
Error	*40*	*17.6*	*0.44*	

IV. Discussion

Embryo culture methods offer new refined ways to characterize the development of embryo and to use embryos in plant improvement programme. The embryos are usually characterized as globular, heart, torpedo and walking-stick type based on their shapes. The immature torpedo shaped embryos (size range 451-700 µm; 22 days old) and mature walking-stick type (26 days old, >700 µm) embryos of five *B. rapa* varieties namely, Safal, Agrani, BINA Sarisha-6, BARI Sarisha-6, BARI Sarisha-15 were tested for their regeneration potentiality. This study observed induction of calli from immature embryos and plantlets from mature embryos.

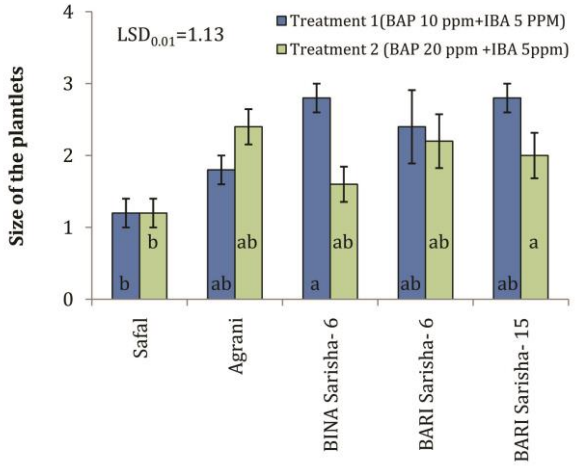

Figure 06. Bar graph showing the size of plantlets (cm) regenerated from mature walking-stick type (26 days old, >700 µm) embryos of five different *Brassica rapa* varieties namely, Safal, Agrani, BINA Sarisha-6, BARI Sarisha-6 and BARI Sarisha-15 when cultured on basal MS media supplemented with 5 ppm IBA and with two different concentrations (10 and 20 ppm) of BAP. Data presented as mean (n=5) ± SE. LSD indicates least significant differences and different letters on the bars indicate statistically significant difference as determined by DMRT analysis.

Immature embryo culture

The immature torpedo shaped embryos were cultured in basal MS media fortified with 1 mgL^{-1} BAP, 0.5 mgL^{-1} NAA and 1mgL^{-1} 2,4-D which has induced calli (Figure 07). It agrees with the report that immature zygotic embryos had significantly greater embryogenic potential than mature embryos (Burbulis and Kupriene, 2005). In the torpedo stage, the embryo has basal cell and suspensor which disappears in mature stage where separate cotyledon can be easily seen. These torpedo shaped embryos have produced calli at 12 to 16 days after inoculation. Sionget et al. (2011) also observed whitish-creamy and yellowish calli in *Brasscia* embryos within 2-3 weeks. Immature torpedo shaped, walking stick type and early mature zygotic embryos of *B. rapa* also responded similarly at 14-21 days in terms of callus induction (Maheswaran and Williams, 1986). Successful induction of calli was also observed from early globular embryos of *B. juncea* when cultured in a double layer culture system (Liu et al., 1993b). Filament culture, using the filaments of varieties Safal and Agrani, produced calli more quickly i.e., within 8.67 and 7.15 days, respectively compared to the response of immature embryo

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culture in these varieties (Alam et al., 2009). Filament culture of BARI Sharisha-7 induced callus even more quickly (within 7 days) as observed from the study of Bhuyan et al. (2007).

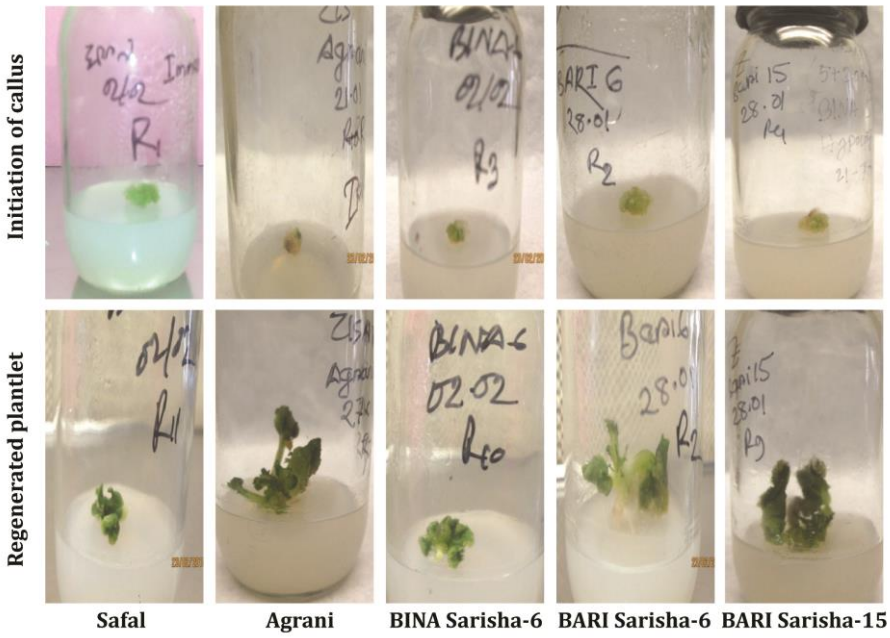

Figure 07. Images (top row) showing the initiation of callus from the immature torpedo shaped embryos (size range 451-700 µm) of five *Brassica rapa* varieties namely, Safal, Agrani, BINA Sarisha-6, BARI Sarisha-6 and BARI Sarisha-15 when cultured on basal MS media supplemented with 1 mgL^{-1}, 0.5 mgL^{-1} NAA and 1 mgL^{-1} 2,4-D. Data recorded on 10 days after inoculation of explants. Regenerated plantlets of different size (bottom row) of the same five varieties from mature walking-stick type embryos (>700 µm) when cultured on basal MS media supplemented with 1 mgL^{-1} BAP,0.5 mgL^{-1} NAA and 1 mgL^{-1} 2,4-D.

The media components play an important role in *Brassica* embryo culture (Liu et al., 1993a). For example, Rahman et al. (2007), in their experiment with varieties of *B. juncea* and *B. napus* observed the quickest (7.5 days) callus induction in the variety Tori-7 usingB5 media whereas Sayem et al. (2006) reported even quicker (6.2 days) callus induction in the same variety. However, they have used MS media supplemented with 2,4-D, NAA and $AgNO_3$. It is however, not clear if the difference in explants such as filament for the former study and leaf segment for the later study is the reason behind this variation in callus induction ability or the observed variations are due to variation in hormonal supplementation.

In general, the older an embryo, the simpler its nutritional needs and hence, the culture of immature embryo is still a challenge (Raghavan and Srivastava, 1982; Rao and Sita, 1996). This study used 1 mgL^{-1} BAP, 0.5 mgL^{-1} NAA & 1 mgL^{-1} 2,4-D for callus induction along with the basal MS media. In tissue cultures, auxins are usually used to stimulate callus production and cell growth, to initiate shoots and rooting (George and Rao, 1980; Pua et al., 1989; Zhang et al., 2003). Auxin is used for rooting in different embryo culture studies (Elhiti et al., 2010). The phytohormone, 2,4-Dichlorophenoxtacetic acid (2.4-D) is universally used synthetic Auxin for callus induction and higher callusing efficiency was observed in different crops along with *Brassica* species (Duncan et al., 1985; Islam and Bari, 2014; Sears and Deckard, 1982). The lowest concentration of 2,4-D that produced callus from immature embryos was reported as 0.5 mgL^{-1} using basal MS media (Islam and Bari, 2014).

The first-generation synthetic cytokinin6-Benzylaminopurine, benzyl adenine or BAP elicits plant growth and developmental responses. BAP was successfully used in various callus induction and shoot proliferation media to see the *in vitro* potentiality of various *Brassica* genotypes (Burbulis et al., 2012; Khan et al., 2010; Sayem et al., 2010; Roy et al., 2010). NAA (1-Napthalene Acetic acid), is usually used

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as a rooting agent which is also used for the vegetative propagation of plants from stem and leaf cutting. Murata and Orton (1987) found that the calli were induced most frequently in Murashige and Skoog (MS) medium with 1.0 mgL^{-1} 2,4-D when NAA is used with this media composition. Sucrose has been widely used as an osmoticum as well as a carbohydrate source in anther and microspore culture and was considered as an essential medium component for the induction of embryogenesis in *Brassica* (Ferrie et al., 1995; Robin et al., 2005a).

Mature embryo culture

Mature walking-stick type (26 days old, >700 µm) embryos of five genotypes of *B. rapa* species were cultured which directly gave plantlets within 7 to 12 days (Figure 05). Maheswaran and Williams (1986) reported primary and secondary embryogenesis from the mature embryos of *B. campestris*. In winter wheat, early mature embryos have shown higher frequency of callus induction and regeneration while the mature embryos directly regenerated plantlets (Özgen et al., 1998). They had reported that on B5- and EC6- based media embryoids did not develop beyond the cotyledonary stage. Burbulis and Kupriene (2005) transferred the cotyledonary stages mature embryo in B5 media and cotyledonary embryos developed into morphologically normal plantlets at a frequency from 57.7 % to 87.3 %.

Response of embryo maturity status on plant regeneration

The five *B. rapa* varieties were found to be highly significant on direct regeneration into plantlets from mature embryo (Figure 05). When immature and mature embryos were cultured on the same media, the variety Shafal has shown the lowest callus induction percentages from immature embryo and the highest plantlet regeneration percentages from mature embryos. In contrast to our results, Chi (2000) has found that media composition greatly influenced the embryo regeneration in combination with embryo maturity status. In many crop species, more than 90% mature embryo had been successfully cultured only with basal MS media (Sharma et al., 1996). Our observations in the present study also suggested that 100% mature embryos had showndirect organogenesis. Similar trend of results were also observed in Banana (*Musa* spp.) by Uma and Lakshmi (2011).

Comparison the varietal differences in both mature and immature embryo culture

The effect of varietal differences on callus induction was statistically highly significant. The variety Safal gave the highest percentage of callus in this experiment (Figure 02). The same variety Safal has reported to show the lowest callus induction percentages when it's anthers were cultured *in vitro* along with a number of other *Brassica* species (Alam et al., 2009). The variety BINA Sarisha-6 produced the lowest callus induction. The size, colour and texture of the callus from five *B. rapa* genotypes was statistically significant and the results are consistent with that of Malek et al. (2013). BINA Sarisha-6 and BARI Sarisha-6 required more time than the rest three other varieties. The required time of plantlet formation of five *Brassica* genotypes was also highly significant. We observed that the varieties BARI Sarisha-6, BINA Sarisha-6 regenerated plantlets more quickly (8 days). Robin et al. (2005) observed the highest percentage of shoot regeneration from anther derived calli of BARI sharisha-8 variety within 23 days of inoculation.

Effects of plant growth regulators

Plant phytohormones or the plant grwoth regulators (PGRs) such as 2,4-D, BAP and NAA were used in this experiment for both immature and mature embryo culture. In the same media composition the variety Safal gave the lowest percentage of callus induction and the highest percentage of plantlet regeneration from immature and mature embryo culture. The results were also somewhat similar for the size, colour and texture of the callus. So, it can easily be said that the PGR has no influence on direct plantlet regeneration processes as even the use of 2,4-D in the media, which otherwise influences callus formation as revealed in several studies failed to induce callus from mature embryos. This is further strengthened by the experiement of (Uma et al., 2011) who observed direct plantlet regenration without any PGR. This contrasting results of both cultures indicate the effect of embryo maturity and genotypic variation for the direct or indirect regenration. But statistially significant

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differntial effect of plant growth regulators was observed for all other callus or plantlet regenration parameters.

V. Conclusion

Characterizing the embryo culture from mature and immature embryos of fives *B. rapa* varieties would be very helpful to improve embryo rescue protocols in future. As the torpedo shaped immature embryos induced callus and walking-stick type mature embryos directly regenerated into plantlets therefore the later protocol could be established as a 'rapid regeneration protocol'. However, further research is needed to improve the embryo cultures techniques.

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VI. Reference

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