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Short Communication

IN VITRO CALLUS REGENERATION OF *BRASSICA SPP* THROUGH ANTHER CULTURE

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Abstract

Brassica is an important oil-yielding crop under Brassicaceae family. *Brassica* is the third most important vegetable oil crop in the world, after palm and soyabean oil. But it takes a long time to develop new Brassica cultivars. Haploid production of *Brassica* spp. through anther culture proved to be an important approach of tissue culture. The purpose of this study was to carry out a suitable protocol for happloid production of *Brassica spp.* and observe genotypic variation for callus induction. Three replications were used containing different concentration of 2, 4-D, BAP, Kiniten and NAA. The highest percentage of callus induction (56.2%) was found in CM 800 within minimum days (18 days) and the nature of callus was compact. It indicates totipotency capacity of cell was also influenced by radiation. Future study was necessary to shoot and root induction process for successful speed breeding program.

Keywords: Anther culture, callus, *In vitro* regeneration and *Brassica spp.*

The world is experiencing rising demands for crop as well as global agricultural production that need to be increased by 60-110% to meet the increasing demands and to ensure food security (FAO 2012). Crop improvement through breeding has been the major tool to stable global food supply. To adequately address these food security challenges, high yielding crop varieties need to be developed as a partial solution (Lenaerts *et al.*, 2019). Development of new varieties is time-consuming as it is dependent on generation period of a crop. Mustard (*Brassica napus*), a member of the Brassicaceae family is not out of this time frame. In biology (i.e. genetics) as breeding material is not genetically uniform or "stable" (i.e. plants are not homozygous) until at least 6 to 8 generations (i.e. self-pollination events). Breeders always use new methods and tools to develop new varieties, and accordingly, have long discussed the pros and cons of different breeding methods, especially with regard to the speed of breeding (Forster *et al.*, 2014). Speed breeding or accelerated plant breeding is an emerging strategy among plant breeders to develop new cultivars in short span of time. Basically it focused on most time consuming component of breeding program known as "line fixation" stage. Doubled haploid (DH) populations are produced by regenerating plants by the induction of chromosome doubling from pollen grains, which greatly shortens the line fixation stage because completely homozygous lines are produced immediately (Mishra *et al.*, 2016). In oilseed rape, the doubled haploid method can reduce the time to develop a new cultivar by about 2-4 years compared to the traditional breeding procedures (Cardoza *et al.*, 2006; Alam *et al.*, 2009). Due to the recalcitrant nature of -

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Brassica tissue in vitro, it eluded any notable progress in this regard for a long time. Since the first report of haploid production in 1920s, the DH, both the range of species and efficiency of production, has been greatly improved and is routinely used in many crop breeding programs (Asif, 2013; Li *et al.,* 2013).

The regeneration of plants from tissue culture is an important and essential component of biotechnological research. Tissue culture techniques can play an important role for improvement of genetic variability by initiating variation (somaclonal variation) or mutation at an unusually high rate (Krishna *et al.,* 2016). Anther culture derived haploids have been used to produce homozygous diploids, which accelerate breeding programmes. Anther culture technique is the most viable and efficient method of producing homozygous doubled haploid plants within a short period (Ali *et al.,* 2021). Haploid embryos or doubled haploid plants can also be used in mutation, genetic engineering, biochemical and physiological studies (Razdan, 1993). Therefore, to harvest the diverse merits of anther culture, the present research work was planned to carry out a suitable and reproducible protocol for deals with the current status of primary knowledge on the production of haploids and DHs through mustard anther culture.

The experiment was carried out during January to March 2022 at the Biotechnology Division of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. *Brassica* genotypes viz., BARI Sarisha-18 with its two mutants obtained from 800 and 1000Gy (M_2 population) of ^{60}Co and first filial population of BARI Sarisha-18 × Tori-7 and Tori-7 were used as plant materials for this study. Flower buds were collected at the late uninucleate stage in the morning before 9:00 am from the research field of Plant Breeding Division of BINA, Mymensingh. Collected buds were surface sterilized by submerging them into 70% ethyl alcohol (MERCK, Germany) for two minutes then rinsed for three minutes by sterilized distilled water for three times. Anthers were aseptically removed from flower buds using fine tweezers and inoculated into 8 cm petridishes each containing 10 ml MS medium (Nitish and Nitish, Duchefa) supplemented with 4 mgl^{-1} 2, 4-D and 1 mgl^{-1} BAP. About fifty anthers of each genotype were inoculated into each replication. The whole procedure was carried out in laminar airflow cabinet. The cultured vessels were then marked with permanent marker to indicate treatment after sealing with parafilm. Cultures were maintained in plant growth incubator at 25^0±1°C temperature in complete dark condition for callus induction and checked to record the response. Callus size was measured by millimeter scale.

The research was operated in Completely Randomized Design (CRD) with three replications. Data were recorded on number of anthers showing callus, percent of callus induction and days to callus initiation and callus size and statistically analyzed to confirm the significance of the experimental results. The standard deviation, percentage of response and mean for all treatments were calculated by using MS Excel 2010. The significance and difference between means were evaluated by Duncan's Multiple Range Test (DMRT) at 5% significance level by Statistix 10 software.

Callus induction performances of all the materials are presented in Table1. Firstly considered trait was number of anthers able to initiate callus induction and it was ranged from 9 to 23. This ranged indicated the genotypic variation of the studied materials in terms of totipotency. Results showed that CM800 anthers cultured on medium supplemented with 4 mgl^{-1} 2, 4-D and 1 mgl^{-1} BAP exhibited the significantly highest response (23) for callus induction in minimum days (18) and the maximum percentage of callus (56.2) was also observed at the same supplement addition. Moreover, anthers of BARI Sarisha-18, Tori-7 and CM1000 responded statistically the same for callus induction (17, 16 and 15, respectively). On the other hand, the lowest response (9) was observed in BARI Sarisha-18 × Tori-7. The second parameter was the number of days required to complete callus induction and found significantly different response from studied genotypes. The callus induction days was ranged from 18 to 30 days. The maximum days required for callus induction was obtained from BARI Sarisha-18×Tori-7 followed by Tori-7. BARI Sarisha-18 × Tori-7 required 30 days whereas Tori-7 required 28 days and both of them are statistically similar. The CM100 required 22 days for callus induction which was statistically identical with BARI Sarisha-18 (23 days). The callus induction rate was faster in CM800, that was different from others and it required only 18 days.

Table 1. Response of varieties on callus induction for studied genotypes

Genotype	No. of anthers showing callus induction	Days of callus induction	Callus induction (%)	Nature of callus
CM800	23a	18c	56.2a	Compact
CM1000	15ab	22b	38.1bc	Compact
BARI Sarisha-18 × Tori-7	9b	30a	22.6c	Compact
BARI Sarisha-18	17ab	23b	43.5b	Compact
Tori-7	16ab	28a	40.0b	Compact

N.B: Mean values having common letter in the column are statistically identical and those having different letters are statistically different

The percentage of callus induction was the highest in CM800 (56.2%) followed by BARI Sarisha-18 (43.5), CM1000 (38.1%) and Tori-7 (40%). Callus induction was lowest in cross material of BARI Sarisha-18×Tori-7 (22.6%). It was found that irradiated materials CM1000 and CM800 showed comparatively good potentiality in number of callus growth than other genotypes. Comparatively lower potentiality in callus growth was observed in BARI Sarisha-18 × Tori-7 followed by Tori-7. This finding confirmed that of Javed and Hassan (1992) who noted that callus growth was better in *B. napus* genotype.

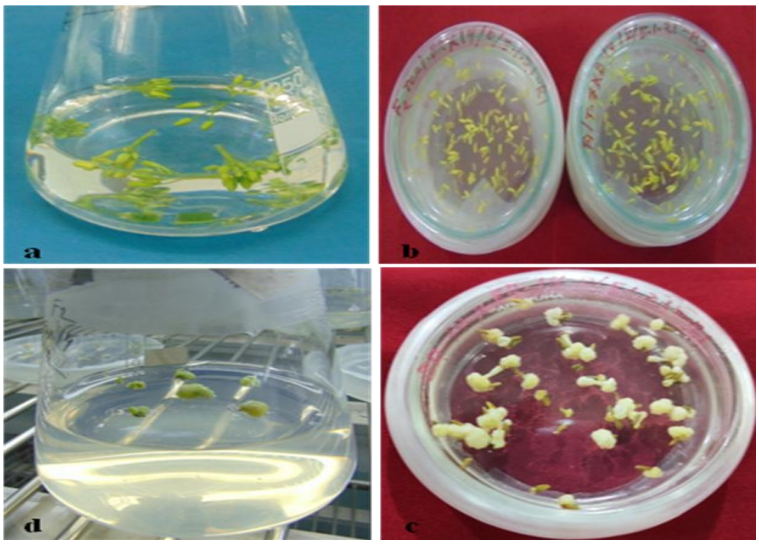

Fig. 1: Steps of anther culture of *Brassica sp*. (a) Collected buds, (b) Anthers on culture media, (c) Callus formation after 21-28 days and (d) Calli on regenerated media

Callus maintenance is essential to get regenerated plants. So MS medium supplemented with 4 mgl^{-1} BAP and 1 mgl^{-1} NAA was used. It was appeared from the study that the responses of calli of different genotypes were different. Among the genotypes, the callus size was found better and identical in CM800 and Tori-7. Callus size of BARI Sarisha-18 and CM1000 was 1.75 mm and 1.70 mm respectively. The lowest callus size was obtained from BARI Sarisha-18 × Tori-7 (1.3 mm).

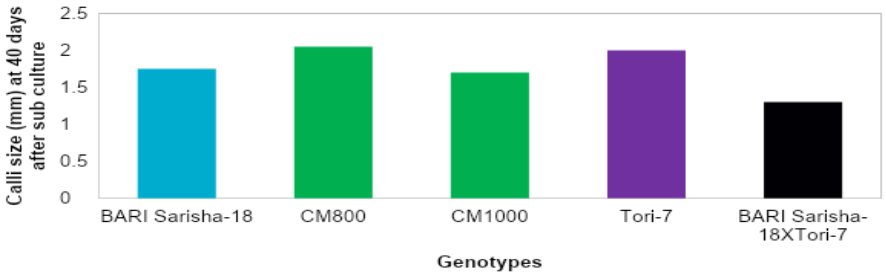

Fig. 2: Influence of genotype on callus proliferation after 45 days of sub culture.

From the above results it indicates that irradiation of *Brassica* genotyoes have positive role to influence the tot potency of calli to regenerate identical plant type. For haploid plant production it can be widely used after suitable optimization of media concentration. Here only two different doses of ^{60}Co were used, it will be better to used more doses for strong recommendation. If we consider the genotype then *B. napus* have more response than *B. campesties*.

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