Journal of Applied Horticulture, 21(3): 178-181, 2019
DOI: https://doi.org/10.37855/jah.2019.v21i03.30
Journal of Applied Horticulture
ISSN: 0972-1045

Improvement in tissue culture-assisted induction of double haploidy in brinjal (*Solanum melongena* L.)

Anjanabha Bhattacharya*, Yogesh Sonone and Bharat Char

*Mahyco Research Centre, Maharashtra Hybrid Seed Company Private Limited, Aurangabad-Jalna Road, Dawalwadi, Jalna, Maharashtra, 431203, India. *E-mail*: *anjanabha.bhattacharya@mahyco.com*

Abstract

Brinjal is a popular vegetable crop in India and widely used in culinary, pickle and industrial purposes. The double haploid technique is used in vegetable crop improvement; thus, reducing lengthy conventional breeding timelines to develop new improved varieties. A study was conducted to develop a robust double haploid (DH) protocol in brinjal. Six genotypes and six media combinations were tested. Two media types showed DH production in brinjal ranging from 0.34-9.27 %. Consequently, MSB-6 is recommended media for brinjal DH in light of the obtained results. Similarly, five out of six genotypes showed DH responsiveness. The genotype-wise responsiveness to anther culture ranged from 2.29 - 7.40 %. DH technique complements the plant breeder's effort to bring new varieties in the shortest time. Therefore, the relevance of improvement in tissue culture assisted double haploidy and continuous improvement of available protocols remains relevant even after six decades of its original discovery.

Key words: Brinjal, double haploid, tissue culture, anther culture

Introduction

Brinjal is a very popular vegetable in India. It is also known as eggplant, aubergine, guinea squash, and belongs to family Solanaceae (Kashyap *et al.,* 2002). Climate change accompanied by global warming is happening at an ever alarming pace and is here to stay. Therefore, food production challenges to feed our ever-burgeoning population, which is expected to rise to 9.7 billion by 2050, will remain a steep challenge. Nutritional security, along with food security, is must and is a national priority. Brinjal is fairly low in calories, loaded with B-complex vitamins and is a good source of iron, copper, manganese and potassium. A useful technique to enhance breeding success is double haploid (DH). The technique of producing haploids was first demonstrated in *Datura stramonium* by Guha and Maheswari in 1966. Soon afterward, it became apparent that this technique has significant relevance in shortening plant breeding program time, which otherwise would take several years (typically 6-7 years).

Though a combination of marker-assisted selection (MAS) with conventional breeding can reduce timelines by a few years, this approach is expensive in terms of instruments, expertise required and, first time application in new crop entries take considerable effort. Further, MAS plants will not represent completely fixed lines whereas DH helps to fix traits in one generation. Therefore, the relevance of tissue culture assisted double haploid remains relevant even after nearly six decades of the original discovery (Veilleux, 1994). DH studies in brinjal were reported several workers (Raina *et al.*,1973; Yadava *et al.,* 1989; Khatun *et al.,* 2006; Mir *et al.,* 2008; Kadhim 2006, Bayas *et al.,* 2011 & 2012). Basay *et al.* (2011) found DH efficiency in brinjal to vary between 1.3-5.3 % in six genotypes under study. Thus, continuous improvement in the brinjal DH protocol is useful to enable its adaptation to a diverse range of germplasm.

A study was conducted in the tissue culture laboratory, Mahyco Research Centre, Jalna, to evaluate various conditions and media compositions affecting brinjal DH. The primary aim of this study is to improve the existing know-how on brinjal DH. All six entries were selected purely to develop a robust DH protocol.

Materials and methods

Plant material: In house, six donor Brinjal entries, designated BDH-104 to 109, were included for developing DH protocol. Seeds were first surface sterilised and grown in controlled growth room (21°C ± 2 °C) with a relative humidity of sixty per cent. Natural light was supplemented with 16 h of fluorescent illumination (195 mmol m^2/s TLD/58W 35V "Daylight" fluorescent tubes; Phillips) with day and night temperatures of 25°C ± 1°C. The light intensity of 10000Lux was maintained with 16 h light/ 8 h dark condition. The donor material was planted in pots in batches so that a continuous supply of flower buds was available for the study. Care was taken to collect flower buds from the first two flushes only as later flushes may have tendency towards recalcitrance to DH due to advancing age of donor plants (Dunwell, 1985). Also, it was ensured that donor plant remained pesticide-free, as chemicals sprayed on plants or applied in soil adversely affects quality of flower buds.

Flower buds: In the initial stage of the study, closed flower buds were carefully classed based on the stage of the microspore development stage. Amenable anthers considered conducive for DH contains uni-nucleate microspores (Harberle-Bors, 1985). The staging was done under microscope with acetocarmine staining. Later only specific size of buds (14-16 mm long) was selected for protocol development experiments. Only fresh healthy buds were collected (Dunwell, 1985) and processed immediately on the media.

Media: The media composition consisted of MS salts (Murashige and Skoog, 1962) with a combination of auxin (NAA) and cytokinin (Kinetin or BAP). Media composition developed was primarily focussed on inducing direct organogenesis, thus avoiding callus induction, which might harbour maternal (somatic) cells in addition to gametic cells. The media was changed every four weeks.

Sterilization of donor flower buds: Fresh flower buds (14-16 mm) were collected in the morning hours (9 AM-10 AM) and surface sterilized for 10min in 0.1 % $HgCl_2$ solution. The buds were thoroughly washed thrice with sterile distilled water to ensure no residue of $HgCl_2$ was left on the explants. Subsequently, the buds were surface-sterilized on Whatmann filter paper under laminar hood.

Anther culture: The buds were placed in a sterile Petri dish. Anthers were excised from the closed flower buds with the help of a surgical scissor. The anthers were carefully transferred to the solid nutrient media with the help of forceps. Sufficient care was taken to ensure anthers were not injured during the plating process as that may result in callus development from anther cell wall somatic tissues. The media is listed in Table 1. The pH of the media was maintained at 5.8. Twenty anthers were placed on each Petri dish (60 x 15 cm^2).

Embryo induction and plantlet regeneration: Petri dish with anther was kept at 4 °C in dark for 5 days and then transferred to 25 °C in dark. Embryo induction was observed after 45 d of anther plating depending on the genotype as evident from small white protrudes. The embryoids were transferred to plain MS media under standard GH light conditions until embryoids germinated and attained the desired size.

Plant acclimatisation: Plantlets were transferred into soil cups for hardening. These cups were covered with plastic bags. Gradually the bags were cut open from corners, to expose plants to the immediate environment. Once plants were found acclimatised, they were moved to net house/greenhouse in individual pots.

DH seed collection: The flowers were self-pollinated and carefully bagged. Fruits were harvested and seeds collected.

Grow-out test: To test success of the DH protocol, DH seeds were planted in the field and checked for uniformity within the lines. Any off-types (not uniform) were removed. From a breeding stand point, this step helps to evaluate the developed DH line and select for stable lines at the same time, bypassing other costly laboratory based confirmative techniques.

Colchicine treatment: The roots of the primary DH plantlets were pruned and dipped in 0.01 % colchicines solution for 4 hours under sunlight (in the morning hours), for greater imbibition ability. The plantlets were then thoroughly washed with sterile water and excess moisture was removed by placing plants in tissue paper towels. Subsequently, plants were transferred to cups in controlled growth chambers. These plants were acclimatised and transferred to the greenhouse for fruiting and seed set.

Results and discussion

Embryoid formation: Depending upon the availability of anthers and genotype responsiveness, 830-2705 anthers were plated on the culture media. Of all the six genotypes used for protocol development, only one genotype remained unresponsive. Previous DH studies involved use of charcoal in the medium as this stimulates androgenesis in pepper (Ciner and Tripirdamaz, 2002) and $AgNO_3$ induced shoot regeneration in cotyledon tissue culture of *Brassica campestris* (Du *et al.,* 2000). Media composition and improvement over known protocols are shown in Table 1. A total of six different media were used in the study. Two out of the six media compositions showed promising results. The

Table 1. Details of genotype and media combination used in developing robust DH protocol in brinjal

Media/genotype	BDH-104	BDH-105	BDH-106	BDH-107	BDH-108	BDH-109	Media-wise total number of anthers cultured	Media-wise number of H/DH plants developed	Media-wise percent anther to plant regeneration
MSB-1	100	140	190	180	120	130	860	00	00
MSB-2	120	140	170	160	140	140	870	00	00
MSB-3	120	140	175	180	130	140	885	00	00
MSB-4	120	130	180	170	110	120	830	00	00
MSB-5	100	150	180	170	150	120	870	3	0.34
MSB-6	400	500	455	490	430	430	2705	251	9.27
Genotype-wise total number of anthers cultured	960	1200	1350	1350	1080	1080	7020	-	-
Genotype-wise number of H/DH plants developed	00	37	35	45	57	80	254	-	-
Genotype-wise percent anther to plant regeneration	00	3.8	2.29	3.33	5.27	7.4	3.61	-	-

MSB-1- MS salts + Vitamins + NAA-2mg/L + BAP-2mg/L + 3 % Sucrose + 0.8 % Agar (PH-5.8) (Khatun *et al.,* 2006 with modifications)

MSB-2- MS salts + Vitamins + NAA-2mg/L + BAP-1mg/L + Zeatin-1mg/L + $AgNO_3$- 5mg/L + 3 % Sucrose + 0.8 % Agar (PH-5.8) (Khatun 2006 *et al.,* with modifications)

MSB-3- MS salts + Vitamins + Kinetin-1mg/L + BAP-1mg/L + 3 % Sucrose + 0.8 % Agar (PH-5.8) (Matsubara *et al.,* 1992 with suitable modifications)

MSB-4- MS salts + Vitamins + NAA-2mg/L + BAP-1mg/L + 2-4 D- 0.5mg/L + 3 % Sucrose + 0.8 % Agar (PH-5.8) (Kumar *et al.,* 2003)

MSB-5- MS salts + Vitamins + NAA 5mg/L + BAP 1mg/L + 3 % Sucrose + 0.8 % Agar (PH-5.8) (Khatun *et al.,* 2006 with modification)

MSB-6- MS salts + Vitamins + NAA-5mg/L + BAP-2 mg/L + 3 % Sucrose + 0.8 % Agar (PH-5.8) (with modifications)

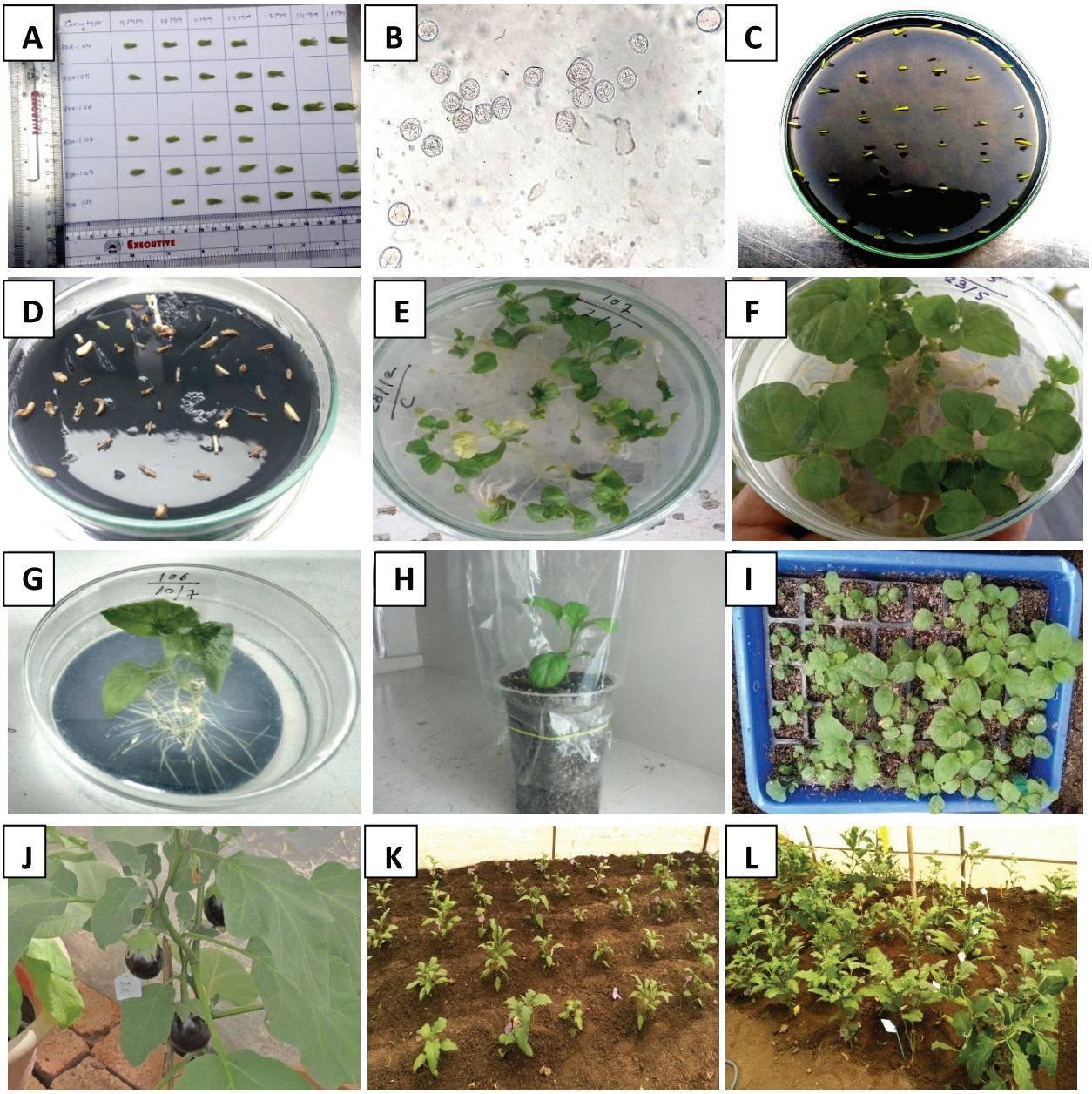

Fig. 1. Brinjal double haploid developmental stages - (A- Brinjal bud length, B- Microspore staining (Acetocarmine), C- Anther culture, D- Embryoid induction, E- H/DH shoot induction, F & G- Brinjal H/DH plants, H- H/DH plant hardened in cup, I- H/DH plants hardened intray, J- H/DH plant hardened in pot, K & L - H/DH plants hardened in Greenhouse/Net-house.

most promising, MSB-6, showed DH plant regeneration at the efficiency of 9.27 %, and MSB-5 showed 0.34 % regeneration efficiency. Studies have earlier hinted that the addition of $AgNO_3$ significantly increased shoot regeneration. The entire workflow of brinjal DH generation is shown in Fig. 1. Plants regenerated from anther culture varied among genotypes in numbers. While BDH-104 remained unresponsive, all other genotypes tested showed 2.29-7.4 % regeneration efficiency. The results clearly show vital relationship between cultivar and media composition.

The success of brinjal DH, depends on various parameters including genotype, age of explants, growth conditions. Our experience with vegetable DH shows that growing donor material in growth chamber gave better results in chilli and cauliflower than field grown materials. Thus, brinjal donor plants were also grown in growth rooms. Genotype recalcitrance could impede universal acceptance of DH technology and limit its acceptance among plant breeders. One way to overcome recalcitrance is by making crosses among responsive and non-responsive ones (Basay and Ellialtioglus, 2013). Promising results were also found in other studies (Basay and Ellialtioglu, 2013). Flower bud staging is an important step as this prevents frequent restaging, and the buds at the same time contain microspores at the preferred stage when plants were grown in a controlled environment. Anther culture is beneficial over microspores, as multiple microspores within anther sac are exposed to cell wall factors, which facilitates pollen-embryo development (Pelletier and Ilami, 1972; Raghavan, 1978). Several studies have shown DH efficiency is related to genotype (Basay and Ellialtioglu, 2013).

To conclude, the results from the present study shows improved DH plant generation compared to published studies on brinjal *viz.*, direct organogenesis while continuously maintaining controlled doner plant materials in a regulated environment, greatly improved DH development. Further, we were able to bypass the

callus stage. The results from the perspective of plant regeneration are addition to the existing know-how on brinjal DH.

References

Basay, S., V. Seniz and S. Ellianltioglu, 2011. Obtaining dihaploid lines by using anther culture in the different eggplant cultivars. *Journal Food, Agriculture and Environment,* 9: 188-190.

Basay, S., S. Sebnem and S. Ellialtioglu, 2012. Effect of genotypical factors on the effectiveness of anther culture in eggplant (*Solanum melongena* L). *Turkish Journal of Biology,* 37: 499-505.

Guha, S. and S.C. Maheswari, 1966. Cell division and differentiation of embryo in the pollen grains of Datura *in Vitro*. *Nature*, 212: 97.

Ciner, O.D. and R. Tripirdamaz, 2002. The effect of cold treatment and charcoal on the in vitro androgenesis of pepper (*Capsicum annuum* L.). *Turkish Journal of Botany*, 26: 131-139.

Du, H., D.H. Zhuang and H. Huang, 2000. Stimulation effect of silver nitrate on shoot regeneration in cotyledon tissue culture of *Brassica campestris*. *Journal of Tropical Subtropical Botany*, 8: 109-112.

Dunwell, J.M. 1985. Haploid cell cultures. In: *Plant Cell Culture*: *A Practical Approach*. R.A. Dixon (Ed.), IRL Press, Oxford, UK, pp 21-36.

Heberle-Bors, E. 1985. *In vitro* haploid formation from pollen: A critical review. *Theoretical Applied Genetics* 71: 361-374.

Kadhim, M.I. and A.K. Ali, 2011. Some factors affecting callus and somatic embryogenesis in anther culture of *Solanum melongena* L. *Egypt J. Experimental Botany,* 7: 83-88.

Kashyap, V., V.S. Kumar, C. Collonnier, F. Fusari, R. Haicour, G.L. Rotino, D. Sihachakr and M.V. Rajam, 2002. Biotechnology of Eggplant. *Scientia Horticulturae,* 1846: 1-25.

Khatun, F., M.B. Meah and N.M. Nasiruddin, 2006. Regeneration of eggplant through anther culture. *Pakistan Journal of Biological Science*, 9: 48-53.

Mir, K.A., A.S. Dhatt, J.S. Sandhu and S.S. Gosal, 2008. Genotype, explants and culture medium effects on somatic embryogenesis in eggplant (*Solanum melongena* L.). *Horticulture, Environment and Biotechnology*, 49: 182-187.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiologia Plantarum,* 15: 473-497.

Raina, S.K. and R.D. Iyer, 1973. Differentiation of haploid plants from pollen callus in anther culture of *Solanum melongena* L. *Z. Pflanzenzucht,* 70: 275-280.

Matsubara, S., K. Hu and K. Murakami, 1992. Embryoid and callus formation from pollen grains of eggplant and pepper by anther culture. *Journal of Japan Society of Horticulture Science,* 61: 69-77.

Pelletier, G and M. Ilami, 1972. Les facteurs de androgenese. In Vitro chez *Nicotiana tabacum*. Z Pflanzenphysiology, 68:97-114.

Raghavan, V. 1978. Origin and development of pollen embryoids and pollen cultures in cultured anther segments of *Hyoscyamus niger* (henbane). *American Journal of Botany,* 65: 984-1082.

Veilleux, R.E. 1994. Development of new cultivars via anther culture. *Hort Science,* 29: 1238-1241.

Yadava, N.R., T.M. Verghese, D.R. Sharma, 1989. Morphogenetic studies in androgenic callus of *Solanum melongena* L. *Current Science,* 58: 637-639.

Received: January, 2019; Revised: March, 2019; Accepted: March, 2019