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Tissue culture interventions in soybean production: significance, challenges and future prospects

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ABSTRACT

Glycine max, commonly known as Soybean is a legume crop which is consumed globally. Due to its high nutritive content, soybean is considered as a cheaper source of complete protein that can address the problem of malnutrition in the poorer sections of society. Soybean can also fulfill the demand of vegetable oil which, at present, is being met through imports. However, need of large cultivation area and suitable climatic conditions restrict the production of high quality and high yield soybean varieties. In this scenario, plant tissue culture techniques such as embryo culture, somatic embryogenesis, organogenic differentiation and protoplast culture can be utilized for mass propagation of superior quality soybean in a limited space and free of seasonal constraints. This paper reviews the scientific aspects of the studies conducted on tissue-culture based propagation of soybean, and the need for ground-level application of these studies for the benefit of both growers and consumers.

Key words: *Soybean, Protein content, Plant tissue culture, Embryo culture, Embryogenesis, Organogenesis, Protoplast culture*

Introduction

Soybean (*Glycine max* L. Merril) is globally the most important and oldest seed legume, that contributes to about 25 per cent of the global edible oil and about two-thirds of the world's protein concentrate for livestock feed. With about 40 per cent protein and 20 per cent oil, soybean is an exceptionally valuable oilseed crop. It produces three times more protein as compared to major cereals crop like wheat, rice or maize (per hectare production). The food derived from soybean provides several health benefits and is a cheaper source of high-quality protein. Soybean oil is the single most important vegetable oil, accounting for 20 per cent of global vegetable oil production. Soymeal accounts for over 60 per cent of world output of vegetable and animal meals and occupies a prominent position among protein feedstuffs used in the production of feed concentrates. Soybean meal is a valuable ingredient in formulated feed for poultry and fish. Soybean meal is a valuable ingredient in formulated feed for poultry and fish. Globally, soybean is considered to be meat, milk, cheese, bread, oil and has earned epithets like "Cow of the field" or "Gold from soil" (Horvath, 1926). Owing to its amino acids composition, unsaturated fatty acids (about 85 per cent), essential fatty acids such as oleic acids (23 per cent), linoleic acid (53 per cent) and linolenic acid (7 per

Abbreviations
PGR – Plant Growth Regulator, MS - Murashige and Skoog medium, B5- Gamborg *et al*. medium, BAP - 6-Benzylaminopurine, Kn- Kinetin (6- furfuryl amino purine, NAA - 1-Naphthaleneacetic acid, IAA- Indole-3 acetic acid, IBA- Indole 3 Butyric acid, 2,4-D- 2, 4-Dichlorophenoxy acetic acid

cent) the protein of soybean is called a complete protein. (Fehr *et al.*, 1992). The crop, therefore, has the potential to eliminate protein malnutrition prevailing in poor sections of society.

Soybean production

Soybean is cultivated in warm and moist climatic conditions. The crop is largely grown in monsoon season from June to October. The Optimum temperature 30-33 °C. Crop can be grown in areas receiving around 600 – 650 mm rainfall. Day length is the key factor in most of the soybean varieties as they are short day plants and are sensitive to photoperiods (Yang *et al*., 2019). Most of the varieties flower and mature quickly if grown under conditions where the day length is less than 14 hours and temperatures conditions are favourable. Soybean can be grown on variety of soils ranging from light to black cotton soils. Land should be well levelled and be free from crop stubble. The sowing should be done in lines 45 to 60 cm apart with the help of seed drill or behind the plough. Plant to plant distance should be 3-5 cm. Usually the crop is rainfed but in situation of early withdrawal of monsoon rains, irrigation is found to be beneficial. When soybean plants mature, they start dropping their leaves. The maturity period ranges from 90 to 140 days depending on the varieties. When the plants reach maturity, the leaves turn yellow and drop and soybean pods dry out quickly. Harvesting can be done by hand, breaking the stalks on the ground level or with sickle. Threshing can be done either with the mechanical soybean thresher or some conventional method as used in other legumes.

In India, soybean was introduced from China in tenth century AD through the Himalayan routes, and also brought in via Myanmar by traders from Indonesia. After cereals, oilseeds are the second largest agricultural commodity, accounting for the 13 per cent of the gross cropped area in the country. As per Directorate of Vanaspati, Vegetable Oils and Fats; Soybean contributes 42 per cent and 20 per cent of the total oilseeds and edible oil production of the country and earns valuable foreign exchange by exporting soya meal. However, the country is meeting its edible oil demand by importing almost 50 per cent of its requirement. Soybean has been traditionally cultivated on a small scale in hilly areas of Himachal Pradesh, Uttarakhand, eastern Bengal, Khasi Hills, Manipur, Naga Hills, and parts of central India covering Madhya Pradesh, Maharashtra and Rajasthan (Agarwal *et al*., 2013). Sadly, overall soyabean production, especially in mountain agriculture is in steady decline (5%–30%) over the past few decades. In traditional hill cropping systems, farmers use soybean, corn, proso millet, amaranth, Brassica spp, etc. as mixed crop. These types of traditional cropping systems are not much advantageous as the crop is grown without any definite proportion or pattern. Soybean cultivation, therefore, is mostly practiced in traditional subsistence farming only to meet the domestic needs of the farmer's family.

Consequently, there is a need of application of advanced agricultural/ biotechnological methods which would to assist in an organized soybean crop production in the country.

Tissue-Culture based propagation strategies for Soybean

Plant tissue culture refers to the technique of culturing plant cells, tissues, organs under controlled nutritional and physicochemical conditions *in vitro* (Thorpe, 2007). The technique exploits the "totipotency" of plants which enables the cells and tissues to develop into complete plant body and express entire genome .The technique is widely used for large scale multiplication of plants (a single explants is capable of producing hundreds and thousands of plants) in a relatively short time period and space, irrespective of season on a year round basis (Akin-Idowu *et al*., 2009). Besides proving its significance in conservation of RET species and production of commercially important plants, plant tissue culture techniques constitute a crucial part of modern agriculture. It has helped in production of disease free, superior quality, homozygous, genetically uniform plant material and in development of new varieties and hybrids. In case of soybean, a myriad of research work across the globe has been conducted on *in vitro* propagation. Discussed below are the different strategies of plant tissue culture that have been adopted by different workers and their significance in soybean production.

Seed/ Embryo culture

Seeds are commonly used explants for micropropagation of crop species. They are easier to collect, store, transport and manipulate under *in vitro* conditions (viz. handling during surface disinfection). A seed houses a zygotic embryo within the

nutritive reserve called endosperm. The embryo itself can be used as an explant for micropropagation studies. Embryo culture is an excellent system for propagation of recalcitrant plant species, rare hybrids and rare wild species. The technique has also been found to be useful in breaking seed dormancy and shortening of breeding cycle of important crop plants. This is attributed to the fact that embryo is an extremely juvenile tissue that exhibits immense morphogenetic potential in vitro. Embryo culture has been successfully tried in soybean by several workers using immature/ mature seed as explants (Table 1).

The studies suggest that different genotypes and cultivars behave differently when treated *in vitro* and one needs to standardize the medium and PGRs to cultivate explants from each mother plant.

Somatic embryogenesis

Somatic embryogenesis is a pathway of plant regeneration *in vitro* where explants are induced to develop into embryos. Since these embryos are somatic in origin, they are called somatic embryos and while their developmental path parallels that of normal zygotic embryos. Somatic embryogenesis is, therefore, a unique technique in plant tissue culture where a somatic cell/tissue can give rise to a zygotic tissue. The main advantage of this method is development of somatic embryos which mimic the zygotic embryos and if encapsulated, mimic a seed. Artificial seeds formed after encapsulation of somatic embryos in a gel-based coating offer advantages like ease of transportation, storage, large scale propagation, minimal periodic maintenance and complete plant development in a short span of time (since one somatic embryo will directly give rise to one plantlet). Use of bioreactors for large scale production and encapsulation of somatic embryos has further proven to be extremely advantageous for large scale clonal plant propagation. Besides, small size and rich cytoplasmic content of globular embryos make them most suitable material for plant cryopreservation (storage of tissues at ultra low temperature of liquid nitrogen); aiding in long term preservation of the species (expanding over years) with minimal laborious maintenance.

However, establishment of somatic embryos is a challenge in itself. Some species have pre embryogenic determined cells which causes easy somatic embryo development *in vitro;* in some species embryogenesis has to be induced while in some cases somatic embryogenesis has not been reported till date. This restricts the application of somatic embryogenesis to a limited number of plant species. Experiments conducted over a considerable time span have shown that *Glycine max* responds favourably towards somatic embryo production (via cotyledonary explants) (Table 2) and can be suitably exploited for embryogenesis related benefits.

It can be inferred from these studies that somatic embryo formation differs in terms of media requirements with each genotype. Komatsuda *et al*. (1991) in their study emphasized that different soybean genotypes require different sucrose concentrations in the medium (modified MS medium with B5 vitamins) for effective somatic embryo development. The findings were reiterated by Dinkins *et al*. (2001) who confirmed in their study that soybean cultivars growing in diverse locations responded differently towards *in vitro* manipulations for somatic embryo development via seed culture. It, therefore, needs to be understood that somatic embryo development varies with different genotypes and different lines have to be screened for efficient somatic embryo development.

Although somatic embryogenesis offers several applications, a major challenge in use of somatic embryos is their low conversion rate. Normally, only a few somatic embryos convert into complete plantlet. The reason is poor maturation phase in embryo development where essential stress tolerant proteins and reserves fail to get accumulated in the embryo. Ackerson (1984) and Tian and Daniel (2000) in their study showed that if soybean somatic embryos are treated with appropriate amount of

Table 1. *In vitro* studies on embryo culture in *Glycine max*.

Nutrient Medium used	References
MS medium	Shan *et al*., 2005; Vural 2010; Phat *et al*., 2015; Rathod *et al*., 2017; Begum *et al*., 2019
B5 medium	Kim *et al*., 1990; Younessi *et al*., 2015; Sojkova *et al*., 2016; Raza *et al*., 2017
Combination of MS and B5 medium	Zia *et al*., 2010; Mariashibu *et al*. 2013; Soto *et al*., 2013

abscicic acid, it leads to improvement in embryo development and maturation. Abscisic acid acts as a stress hormone, helping the embryo to tide over unfavourable conditions and ensuring better plantlet formation when conditions are suitable.

Indirect organogenesis

Indirect organogenesis refers to *in vitro* organ (shoot/root) formation from explants through an intermediary callus phase. Callus refers to the undifferentiated mass of cells induced from a differentiated tissue. In this method, the explant is first induced to undifferentiate into a mass of unorganized cells (callus) and then directed to form a differentiated organ (e.g. shoot) which is further multiplied and propagated into complete plantlet. The benefit of this method includes rapid plant propagation and development of new varieties by somaclonal variations. These variations are caused by *in vitro* culture conditions provided to the undifferentiated cells which induce genetic variations in the regenerated tissues. Somaclonal variations are considered an effective method for variety development as it overcomes the need of excess time (as required in breeding methods), complicated procedure and approval for variety release (as in case of plant transgenics). The variety so developed is also easily acceptable as it involves no foreign gene manipulation. However, such variations require a well established system of plant regeneration from callus. In case of soybean, several studies have been done for callus mediated plantlet production.

Brwale *et al*.(1986) studied the efficiency of soybean seeds derived embryos in regenerating plants via callus culture and embryo formation on MS medium containing BAP, NAA. Liu *et al*., (1997) reported an important observation that besides exogenous PGR application,endogenous IAA and polyamine content in soybean explants are crucial in determining effectivity of callus induction and further development *in vitro*. Besides juvelile explants, mature cotyledons and embryos have also been used for callus regeneration in soybean (Joynet *et al*., 2010)

Leaf culture of soybean has been reported to develop salt tolerant varieties of soybean via intermediate callus culture. Wada *et al*. (1981) were able to generate salt tolerant varieties of soybean on medium supplemented with upto 0.1 per cent sodium chloride. They also showed that salt stress could be alleviated by supplementing calcium chloride in the culture medium. This pilot study can be useful in generating salt tolerant varieties of soybean which can be further tested in field for performance under natural conditions. Leaf is considered a preferable explant as its collection does not harm the plant and provides uniform cells for differentiation and rapid plant propagation. Wright *et al*. (1987) had reported a protocol where complete soybean plantlets could be developed from small leaf segments of the mother plant. In their study sequential use of CS23 medium and B5 medium supplemented with different plant growth regulators was reported.

Direct organogenesis

Direct organogenesis refers to direct organ (shoot/root) development from cultured explants. Nodal segments are most commonly used for production of callus-free shoot cultures which develop into plantlets that are clones of the mother plant. In this method, preformed axillary buds present in the nodal segments are induced *in vitro* and further multiplied to develop genetically identical copies of the mother plant. The technique can be very useful for mass production of superior quality soybean cultivars/ hybrids. Hitoshi *et al*. (1980) reported a mass propagation strategy for *Glycine max* using stem node segments. They used sodium hypochlorite solution to surface sterilize the explants and used MS medium, BAP and IBA to stimulate mul-

Table 2. *In vitro* studies on somatic embryogenesis in *Glycine max*

Nutrient Medium Used	Reference
MS medium containing BAP, NAA, IBA	Bonacin *et al*. (2000)
MS medium supplemented with 2,4-D, BAP and IBA	Branch *et al*. (2002)
modified MS medium (containing B5 vitamins) and additives like maltose, activate charcoal	Santos *et al*. (2006)
Modified MS medium	Loganathan *et al*. (2010)
MS medium (containing B5 vitamins) and supplemented with 2,4 D, NAA , BAP and IBA	Hyunh (2015)
MS medium and usage of BAP, NAA and IBA	Islam *et al*. (2017)
D40 medium	Raza *et al*. (2020)

tiple shoot formation. However, considering the fact that nodal explants need to be collected from healthy mother plants and immediately inoculated onto culture medium (before it perishes) and because of advantages that seeds render, seed have been preferred over other explants in case of soybean.

Protoplast culture

Protoplast culture is another important technique in plant tissue culture where protoplasts are isolated from plant cells and cultured in laboratory. Protoplast culture can be very effectively used for development of hybrids (via protoplast fusion) and transgenic cells (by easy uptake of foreign gene by protoplasts due to absence of cell wall). However the challenge is to establish a regeneration protocol of plants from protoplasts. Hammat *et al*. (1987) successfully isolated protoplasts from cotyledons of wild *Glycine* sps. and were able to generate complete plantlets *in vitro* by culturing protoplasts on MS medium containing NAA, BAP and IBA. This was followed by another attempt of regenerating *Glycine* plantlets via protoplasts isolated from seed hypocotyls (Hammat *et al*., 1988). A similar study for development of transgenic soybean was reported by Hinchee *et al*. (1988) using *Agrobacterium* vector. They used seed cotyledon as starting material and cultivated the transformed tissue on RV-5 medium followed by growth on B5 medium. Myers *et al*. (1989) also gave a report on plant regeneration of a wild species of soybean from suspension derived protoplasts. They used seeds as explants for establishment of shoot cultures on B5 and MS medium for their study. The studies show that protoplast culture can be successfully done for soybean plant regeneration. These results can be used as a pre study for protoplast culture of superior cultivars so that rapid formation of hybrids or transgenic soybean could be done.

Plant tissue culture techniques have also been used to establish conditions for development of healthy *Glycine max* plantlets. Iron deficiency chlorosis in soybean has been studied via a tissue culture system on MS medium devoid of any PGRs and modified MS medium containing low concentrations of auxins (Stephens *et al*., 1990). Mosquim and Sodek (1991) performed a study with soybean seeds to generate plants which had a better reserve protein synthesis. They used MSB medium containing NAA, BAP and IBA in their study.

Overall, plant tissue culture can be extremely useful in mass propagation of superior quality soybean plants through different regeneration pathways.

Conclusion and Recommendations

Soybean is a nutritionally rich legume having a good national and international market. Despite the suitable conditions for soybean production, India still imports soybean from other countries to meet the domestic demand. Soybean cultivation is currently being done in scattered parts of the country and calls for adaptation of modern agricultural practices and scientific interventions. Several researchers have reported soybean production using plant tissue culture techniques. However, there have been no concerted efforts towards transfer of this technology from laboratory to the farmers. Our recommendations are:

- Plant tissue culture laboratories must take up micropropagation of soybean cultivars obtained from certified institutes / government bodies. Field tested, superior quality mother plants of soybean should be used for explant extraction and *in vitro* culture establishment. Techniques of clonal propagation like axillary bud culture will result in mass propagation of high quality plants which can be further supplied to the farmers through government bodies or through direct distribution to the growers.
- Callus culture can be tried to develop somaclonal variations in soybean cultivars. The variations so developed can be tested for genetic stability and superiority over the parent plant. If tested positive, the variant can be released as a new variety of soybean. This may include a variety with enhanced nutritive content, biotic/ abiotic stress tolerance etc. The variety can be easily made available to the growers as it does not involve genetic transformation and overcomes extensive procedure for approval from government bodies as in case of a transgenic crop.
- Embryo culture can be used to grow soybean hybrids that have been developed by breeding techniques but are recalcitrant to grow in field. This will ensure that the best varieties created by breeders are made available to the farmers.
- Somatic embryogenesis can be easily done in selected cultivars of soybean. The embryos can

be developed into artificial seeds, stored and transported easily to far off places. A plant tissue culture lab in the target area can then easily generate soybean plantlets from embryos in a single step and supply plantlets to the growers in the area.

- Protoplast culture can be done in soybean varieties for rapid development of new hybrids by fusion and subsequent regeneration *in vitro*. This will provide the farmers with area- specific new varieties

It is imperative that all plantlets developed by plant tissue culture techniques are suitable hardened and acclimatized before being handed over to the growers. Besides, plant tissue culturists must adopt low cost propagation techniques to minimize soybean production cost so that the plant material can be made available to the farmers at minimal possible cost. Once good returns are received, more farmers will take up soybean cultivation as a main crop rather than as a mixed crop. Soybean has the potential to provide nutritional security to a developing country like India and plant tissue culture interventions can be of immense help for sustainable production of this nutritional food crop.

References

Ackerson, R.C. 1984. Regulation of Soybean Embryogenesis by Abscisic Acid. *J Exp. Bot.* 35 : 403-413.

Agarwal, D. K., Billore, S.D., Sharma, A.N., Dupare, B.U. and Srivastava, S.K. 2013. Soybean: Introduction, Improvement, and Utilization in India—Problems and Prospects. *Agric Res.* 2 (4) : 293–300.

Akin-Idowu, P.E., Ibitoye, D.O. and Ademoyegun, O.T. 2009. Tissue culture as a plant production technique for horticultural crops. *Afr. J. Biotechnol.* 8(16): 3782-3788.

Begum, N., Zenat, E.A., Sarkar, M.K.I., Roy, C.K., Munshi, J.L. and Jahan, M. A.A. 2019. *In vitro* micro propagation of soybean (*Glycine max*) BARI-5 Variety. *The Open Microbiology Journal*. 19 : 1874-2858.

Bonacin, G.A., Antonio, O.M. and Oliveira, R.C. 2000. Induction of somatic embryogenesis in soybean: physicochemical factors influencing the development of somatic embryos. *Genetics Mol. Bio.* 23 : 865-868.

Branch, S.R., Wright, M.S. and Tomlin, E.S. 2002. Screening of soybean, *Glycine max* L.) Merrill, lines for somatic embryo induction and maturation capability from immature cotyledons. *In Vitro Cell Dev Biol. Plant*. 38 : 543-548.

Brwale, U.B, Kerns, H.R. and Wildohm, J.M. 1986. Plant regeneration from callus cultures of several soybean genotypes via embryogenesis and organogenesis. *J. Planta.* 167 : 473-481.

Directorate of Vanaspati, Vegetable Oils and Fats. 2019. Directorate of Economics & Statistics. Ministry of Agriculture & Farmers Welfare. Govt. of India.

Fehr, W. R., Welke, G.A., Hammond, E.G., Duvick, D.N. and Cianzio, S.R.1992. Inheritance of Reduced Linolenic Acid Content in Soybean Genotypes A16 and A17. *Crop Sci.* 32 : 903-906

Hammatt, N. and Davey, M.R. 1988. Isolation and culture of soybean hypocotyl protoplasts. *Plant Cell Dev. Biol.* 24 : 601-604.

Hammatt, N., Kim, H.I., Avey, M.R., Nelson, R.S. and Cocking, E.C. 1987. Plant regeneration from cotyledon protoplasts of *Glycine* canescens, G. Clandestine. *J. Plant Science.* 48 : 129-135.

Hitoshi, S., Thanh, H. Voqui-D and Cheng, T.Y. 1980. Stimulation of multiple shoot formation on soybean stem nodes in culture. *J. Plant Science Letters*. 19 : 193-201.

Horvath, A. A. 1926. Changes in the blood composition of rabbits fed on raw soybeans. *J Biol Chem*. 68 : 343–355

http://www.sopa.org/statistics/world-soybean-production/dov 4th June 2020

Hyunh, H.N, Singh, S.K., Lal, S.K and Prabhu, K.V 2015. High frequency regeneration in soybean through direct somatic embryogenesis from cotyledons. *Indian J Plant Physiol*. (20) : 232-239.

Islam,N., Islam,T., Hossain,M.M, Bhattacharjee, B.M., Hossain, M. and Islam, S.M.S. 2017. Embryogenic Callus Induction and Efficient Plant Regeneration in Three Varieties of Soybean (*Glycine max*). *Plant Tissue Cult. Biotech.* 27(1) : 41-50.

Kim, J., Lamotte, C. and Hack, E. 1990. Plant regeneration in vitro from primary leaf nodes of soybean (*Glycine max*) Seedlings. *J Plant Physiol.* 136(6) : 664-669.

Komatsuda, T., Kaneko, K. and Oka, S. 1991. Genotype × Sucrose Interactions for Somatic Embryogenesis in Soybean. *Crop Sci.* 31 (2) : 333-337.

Liu, Z.H., Wang, W.C. and Yan, S.Y. 1997. Effect of hormone treatment on callus formation and endogenous indole-acetic acid and polyamine contents of soybean hypocotyl cultivated *in vitro*. *Bot. Bull. Acad. Sin.* 38 : 171-176.

Loganathan, M., Maruthasalam, S., Ling, Y.S., Wei, C.L., Wen, H.H., Pei, F.L., Chih, W.Y. and Chin, H.L. 2010. Regeneration of soybean (*Glycine max* L. Merrill) through direct somatic embryogenesis from the immature embryonic shoot tip. *In Vitro Cell Dev Biol-Plant*. 46 : 265–273.

Mariashibu, T.S., Anbazhagan, V.R., Jiang, S-Y, Ganapathi, A. and Ramachandran, S. 2013. *In vitro* Regeneration and Genetic Transformation of Soybean: Current Status and Future Prospects, A Comprehensive Survey of International Soybean Research - Genetics,

Physiology, Agronomy and Nitrogen Relationships, James E. Board, IntechOpen, DOI: 10.5772/54268. (https://www.intechopen.com/books/a-comprehensive-survey-of-international-soybean-research-genetics-physiology-agronomy-and-nitrogen-relationships/in-vitro-regeneration-and-genetic-transformation-of-soybean-current-status-and-future-prospects)

Mosquim, P.R. and Ladasiav, S. 1991. Culture of soybean fruit explants: growth conditions and efficiency of nitrogen sources for reserve protein synthesis. *PCTOC.* 27: 71–76.

Myers, J.R., Myers, P.A. and Collins, G.B. 1989. Plant regeneration of wild *Glycine* species from suspension culture-derived protoplasts. *Plant Cell Rep.* 8 : 112-115.

Phat, P., Rehman, S., Jung H. and Ju, H.J. 2015. Optimization of soybean (*Glycine max* L.) regeneration for Korean cultivars. *Pak. J. Bot.* 47(6): 2379-2385.

Rathod, B.U., Dattagonde, N.R., Jadhav, M.P. and Behre, P.P. 2017. Effect of Different Growth Regulators on Soybean (*Glycine max* L.) Regeneration. *Intl J Curr Microbiol. App. Sci.* 6(11): 2726-2731.

Raza, G. Singh, B. and Bhalla, P. L. 2017. *In vitro* Plant Regeneration from Commercial Cultivars of Soybean. *BioMed Research International*. 1-9.

Raza, G., Singh, M.B. and Bhalla, P.L. 2020. Somatic Embryogenesis and Plant Regeneration from Commercial Soybean Cultivars. *Journal of Plants.* 9(1) : 35-38.

Shan, Z. Raemakers,·K. , Tzitzikas E.N., Ma, Z. and Visser R.G.F. 2005. Development of a highly efficient, repetitive system of organogenesis in soybean (*Glycine max* (L.) Merr). *Plant Cell Rep.* 24 (9) : 507–512.

Sojková, J.Z., Gregorová, I., Zimová,Z., Matusikova, M., Mihálik, I., Kraic, D. and Moravcikova, J. 2016. *In vitro* Regeneration Potential of Seven Commercial Soybean Cultivars (*Glycine max* L.) for Use in Biotechnology. *Nova Biotech et Chimica*. 15 : 1-11.

Soto, N., Ferreira, A., Delgado, C. Enríquez, G.A. 2013. In vitro regeneration of soybean plants of the Cuban Incasoy-36 variety. *Biotecnología Aplicada.* 30 (1) : 34-38.

Stephens, P.A, Widholm, J.M. and Nickell, C.D. 1990. Iron-deficiency chlorosis evaluation of soybean with tissue culture. *Theory Appl Genet.* 80 : 417-420.

Thorpe, T. 2007. History of plant tissue culture. *J. Mol. Microbial Biotechnol.* 37 : 169-180.

Tian, L. and Daniel, C.W. 1999. Improvement of soybean somatic embryo development and maturation by abscisic acid treatment. *Canad J Plant Sci.* 80 : 271-276.

Vural, H. C. 2010. Reproduction by seed and tissue culture of Soybean (*Glycine max* (L.) Merr.) Growing in Turkey. *J Appl Biol Sci.* 4 (1): 55-58.

Wright, M. S, Koehler, S. M., Hinchee, M. A. and Carnes, M.G. 1987. Regeneration of soybean (*Glycine max* L. Merr.) from cultured primary leaf tissue. *Plant Cell.* Rep. 6(2) : 83-89.

Yang, W., Wu, T., Zhang, X., Song, W., Xu, C., Sun, S. , Hou, W. , Jiang, B., Han, T. and Wu, C. 2019. Critical Photoperiod Measurement of Soybean Genotypes in Different Maturity Groups. *Crop Sci.* 59 (5) :2055-2061.

Younessi, H., Izadi-Darbandi, A., Malboubi, M.A. and Ebrahimi, M. 2015. High-throughput direct regeneration of Soybean mutant and common lines from cotyledonary node. *J Plant Moler Breed.* 3(1) : 50-60.

Zia, M., Rizvi, Z.F., Riaz, R. and Chaudhary, M. F. 2010. Micropropagation of two Pakistani soybean (*Glycine max* L.) cultivars from cotyledonary nodes. *Spanish J Agri Res.* 8(2) : 448-453.