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Reproduction by Seed and Tissue Culture of Soybean (*Glycine max (L.) Merr.*) Growing in Turkey

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Abstract

Human health is not achievable unless adequate amounts of nutritious and safe foods are available and accessible during all life stages. An estimated one-third of the world's population, largely in the developing world, is currently food and nutrition insecure. The most obvious use of plant products in traditional biotechnology was for food. New and even better varieties were produced when early humans learned to cross-pollinate plants and became plant breeders. Soybean breeding has resulted in significant improvements in yield potential, stability of yield, adaptation of the species to mechanical harvest, and yield protection through improved disease resistance. Due to the nature of plant science agriculture, broadly defined as a manipulation of available plant resources to meet the needs of the growing human population, the environment in which plants are grown for agricultural production continuously offers new obstacles to agricultural production. The present invention provides a tissue culture of regenerable cells from a plant, or parts thereof, produced by growing seed and a soybean plant regenerated from the tissue culture. The present invention also provides a method for developing a soybean plant in a soybean breeding program using plant breeding techniques.

Keywords: Soybean (*Glycine max (L.) Merr.*), Tissue Culture

INTRODUCTION

Glycine max (2n= 40), which belongs to the *Fabacea* (*Leguminacae*) family, is a significant crop in many aspects. Soybean seeds are either flat or round-shaped. The colour of the seed coat is of different tones of yellow or brown. Soybean, which is reported to have been first imported into Turkey in the 1930s, was first grown in the Black Sea region. The production and consumption of soybean (*Glycine max*), which is a summer crop, has increased particularly in the United States and also in many European countries [11]. Soybean has come into prominence with respect to its use for nutritional and health purposes, in the form of soybean oil, soy protein, soy milk or soy flour [1]. Furthermore, soybean has aromatic and industrial use and contains various substances, which are used as raw material for several pharmaceuticals. The significance of soybean has been better understood in recent years, and its cultivation area has increased greatly all over the world. Its seeds contain 18-24% fat and approximately 40% protein. Soybean is known to be capable of the fixation of organic matter and nitrogen in soil. Although it genetically originates from China, recently, soybean (*Glycine max*) has started to be cultivated extensively in America, Europe and Turkey. Producers and breeders have reported that soybean (*Glycine max*) improves soil structure by means of the bacteria found on its roots, which fix nitrogen in soil. Soybean is the second most extensively used biotechnological product in Turkey.

Different results have been obtained in scientific research conducted on soybean (*Glycine max*) in Turkey. Karasu et al. (2002) cultivated 8 different varieties of soybean for 3 years under the conditions of Bursa province, and determined the grain yields for the three year cultivation period. Cinsoy et al. (2005) reported that the yields of the different soybean genotypes they investigated varied. Thompson et al. (1989) investigated the genotype performance of different cultivars and lines of soybean (*Glycine max*). The present study was aimed at the germination of the seed explants of soybean (*Glycine max*) by efficient modified methods, as the yield and quality traits of this crop are influenced greatly by environmental factors, due to its being a short-day plant. In this context, firstly field trials were conducted in the study period, and using the seed explants of cultivars obtained from producers in the Mediterranean region, it was targeted to increase the germination rate of seeds *in vitro,* and to achieve rapid production of seedlings using the tissue culture technique with a view to contribute to farmers and the agricultural economy. Furthermore, the study was supported by molecular genetic methods for seed breeding. Molecular genetic methods based on the use of molecular markers and tissue culture studies have

gained significance, in that they enable the more efficient use of plant breeding programmes for the protection of plant gene resources. Following the collection of plants in field trials, in cases where problems are encountered in the propagation and identification of the crop explants, the determination of genetic diversity of gene resources or in the collection of plant materials, which have persistent seeds that reproduce vegetatively, in vitro techniques, including tissue culture, prove to yield success [14]. In such cases, the plant explants to be cultured must be selected with precaution. When in vitro reproduction methods are used and somatically produced materials are cultured, generally agronomic characteristics should also be taken into consideration. In plant tissue cultures, due to cultures with callus-like organization being prone to somaclonal variation, generally tissues organized similar to sprouts are preferred to be used. In plant breeding programmes, gene transfer, selection and the determination of potential cultivars are also of great significance. Therefore, in the present study, following the selection of appropriate meristems, the tips of the sprouts were obtained, and the roots, stem and leaves of the plant were produced from seed explants in the tissue culture laboratory. The present study was aimed at method validation and the establishment of a modified method for the provision of optimal tissue culture conditions for the germination of the seeds of soybean, which is a protein-rich crop of the Far East, consumed in the form of meat, milk, flour and sauce.

MATERIALS and METHODS

The plant material used in the present study was obtained from producers located in the Mediterranean region, and together with field trials conducted in this region, tissue culture studies were performed in the Archeometry and Biotechnology Research Laboratory of Selçuk University Faculty of Science. The observation of soybean (*Glycine max*) seeds, which germinate under humid and dark conditions, revealed that, firstly the seed coat splits, followed by the sprouting of the rootlets (radicles), which constituted the initial root system, from this split. The downward growth of these rootlets in soil was followed by the formation of the first sprout (Figure 1) [6].

Figure 1. Shoots forming of soybean (*Glycine max (L.) Merr.*) seed

Tissue Culture Studies Medium

One volume of MS medium was used for the culture of sterile seeds.

Culture of the plant material

Sterile seeds ready for culture, were inoculated into petri dishes using a sterile forceps. The inoculated seeds germinated, and it was observed that seedlings with fragmented leaves formed within 21 days (Figures 2 and 3). Strong sprouts taken from well-developed plants were sent to the laboratory, and were first trimmed to a smaller size and then washed in soapy water. Their surface was sterilized by means of agitation in 20% sodium hypochloride solution for 20 minutes. This procedure was performed in a sterile container, and 1-1.5 mm-long tips of the sprouts taken from the last washing solution were inoculated into the medium, such that the tips of the first shoots to develop were used for rooting.

Figure 2. Soybean (*Glycine max (L.) Merr.*) seed

Figure 3. Plantlets status of soybean (*Glycine max (L.) Merr.*)

Rooting Stage

Macroscopic and microscopic identification was performed when the length of the roots of the sprouts reached a level of 10-20 mm. The developmental stages of the soybean seed is given in Figure 4. All tissue culture studies were performed with three replicates. Plant growth regulators, such as *brassinosteroids,* accelerate efficient seed germination and vegetative growth against environmental stress factors during the growth and development of flowering plants, including soybean [15]. In the present study, for the acceleration of seed germination, the MS medium (1-3mg/l) was added indole acetic acid (IAA) and gibberellic acid (GA_3) [9]. Germination trials were controlled. The root, stem and leaf explants of the plant formed within 45 days, followed by the conduct of pot and field trials. Accordingly, it was

determined that the yield of plants germinated and grown under tissue culture conditions was much greater than the potential yield obtained upon the direct sowing of seeds. Gibberellic acid and indole acetic acid used in the present study directly stimulated growth and exhibited a positive influence on yield.

Figure 4. Developmental stages of soybean seed

DISCUSSION and CONCLUSION

Glycine max., which is naturally diploid, due to its vegetative characteristics, is used both as a feed crop and an industrial crop [10]. Tissue culture studies conducted on *Glycine max*. are limited in number with mostly in vitro propagation having been performed [2]. In the present study, in order to obtain explants, seeds were germinated under sterile conditions so that plantlets would be regenerated. Following the sterilization of their surface, seeds were ground or rubbed without any further procedure, and were left to germinate in hormone-free MS medium and on filter paper [5]. No problem was encountered during the germination stage, and seeds were inoculated into hormone-free MS medium leading to the formation of regenerated plantlets, ready for obtaining explants, within a period of 21 days. The findings of the present study demonstrated that the optimization of the tissue culture conditions of soybean, which is a short-day plant and of which the yield and quality are influenced by environmental factors, may contribute greatly to its cultivation as a main crop in Konya province and the reorganization of the production pattern of the region [4]. By means of the tissue culture of seed explants, a new cultivar specific to the species and origin investigated, was obtained for soybean, which is influenced readily by environmental conditions [7]. The MS medium yielded a satisfactory reproduction rate with respect to the viability and number of sprouts under tissue culture conditions. Accordingly, it was concluded that, a satisfactory reproduction yield was obtained from the tissue culture method in the present study, which was aimed at the propagation and culture of soybean. A rooting rate of 80% was achieved with the indicated method. The developed stage of the soybean crop following tissue culture under in vitro conditions is presented in Figure 5.

Figure 5. In vitro conditions, soybean plants after tissue culture

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