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Review Article

EXPLORING THE POTENTIAL OF IN VITRO TISSUE CULTURE IN BREEDING PROGRAMS OF LEGUME AND PULSE CROPS: UTILIZATION AND PRESENT CONDITION

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Abstract: *Legumes are an important family of flowering plants, comprising more than 13,000 species and 600 genera. The term "pulses" refers to dried seed crops cultivated for food, rather than oil extraction. Lentils, chickpeas, cowpeas, mung beans, black grams, and pigeon peas are examples of such pulses, as they are highly nutritious and widely consumed by humans. Also, they can fix nitrogen in the soil with the help of bacteria that live in harmony with them. This makes crop cycles less dependent on chemical fertilizers. Before new genotypes were used, the traditional ways of raising pulses took a long time and were hard to do. So, alternatives based on biotechnology can be helpful in this area. Researchers are investigating the efficacy of tissue culture, regeneration techniques, gene transfer, and transformation methods in pulse crops. The aim is to determine how these approaches can be utilized to improve the production and quality of pulse crops. Also discussed are anther, microspore, embryo, and ovary growth and their possible uses in pulses. The study concludes that in vitro tissue culture is a useful tool for breeding programs of leguminous pulses. It can help make better legume crop varieties, leading to higher yields and better quality*.

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Introduction

The 20,000 species in the Fabaceae family make it the third-biggest plant family in the entire country and the second most important source of food and protein for humans (Weeden 2007; Cannon et al. 2009). The FAO recognizes 11 "pulse crops" in the Leguminaceae family, such as peas, beans, chickpeas, lupins, lentils, cowpeas, mung beans, black grams, and pigeon peas, which are grown only for their grains (Akibode and Maredia, 2011). "Pulse" comes from the Latin word "puls," which means thick soup or mush. More and more people realize that pulses are good for your health because they are a cheap source of proteins, important minerals, vitamins, secondary metabolites like flavonoids, and other nutrients (Cannon et al., 2009). Pulse crops, such as *Pisum sativum* L., *Vicia faba* L., *Lens culinaris* Medik, *Phaseolus vulgaris* L., *Lupinus* sp., and *Cicer arietinum* L. have been heavily researched to increase yield and quality (Pérez de la Vega et al., 2011; Torres et al., 2011; Gaur et al., 2012). The FAO states that the area of pulse crops has grown from 64 million hectares in 1961 to 86 million hectares in 2014. Other types of pulse crops, such as cowpeas, mung beans, black grams, and pigeon peas, are mostly cultivated in warm climates (Smkal et al., 2015). Breeding of fava beans is conducted similarly to other self-pollinating species due to its self-pollinating nature. In Conventional breeding programs, the first step is to find and collect a good genetic variety for the traits that need to be improved.

Then, breeders create new kinds of variation by crossing different genetic resources and using the offspring to make new inbred lines. But this process often gets stuck because there aren't enough genes. This is because pre-breeding efforts don't take advantage of the diversity of wild species. Based on Harlan and de Wet's classical definition from 1971, legumes are put into genetic pools to increase the genetic base and add desired traits. The nitrogen dynamics of six legume species (*Pisum sativum*, *Cajanus cajan*, *Cicer arietinum*, *Vigna unguiculata*, *Lens culinaris*, and *Lupinus* sp.) and two species of Vigna (*Vigna mungo* and *Vigna radiata*) were investigated. The results showed that the six legume species and two Vigna species had primary, secondary, and tertiary pools. However, *Pisum sativum* and *Vigna faba* only had primary and secondary pools. Because of this, the secondary genetic pool can't be used for normal plant breeding. Instead, it has to be used with biotechnology. In-vitro

technologies might be able to solve some of the problems and problems that come with standard ways of breeding. Using in-vitro culture methods in breeding programs for pulses can be helpful in two ways: it can help control genetic variation and speed up the normal breeding process. This review examines how well in-vitro tissue culture techniques can breed pulses and their benefits. Plant breeding has used in-vitro methods for more than 70 years, and there is a lot of literature about them. This review will focus on literature from the last ten years.

Tissue Culture Propagation

The prospect of being able to study the genetic variation in a relatively short period has been provided by the process of in vitro vegetative propagation of plants, which is conducted in aseptic conditions, as documented by Cruz-Cruz et al. (2013). This process also allows for the rapid multiplication and production of plant material. This type of plant growth includes three steps: somatic embryogenesis, the production of adventitious shoots, and the production of axillary shoots from axillary buds and meristems that already exist (Ahmed et al., 2001). Micropropagation, which uses existing axillary buds and meristems, makes it possible to grow elite cultivars from clones on a big scale, which speeds up the breeding process (Deo et al., 2010). Unfortunately, most species of legumes have autogamy and orthodox seeds. This makes these expensive uses not very interesting, so they are rarely done. Still, they can be very helpful in saving wild germplasm that is threatened by habitat loss, like lentils (Sevimay et al., 2005). This is because they can provide disease-free material to keep breeding lines going, shorten quarantine periods, and quickly increase the number of plants collected (Brown et al., 2014). Espósito et al. (2012) showed for peas that F1 hybrid clonal propagation can be used to make enough F2 populations for breeding programs that need crosses that are hard to make.

Somatic Embryonic Formation

Somatic embryogenesis is a process that allows for the development of haploid or diploid somatic cells into various plant species without the need for gamete fusion at any stage of embryological development. This process has two main steps: inducing embryogenesis and expressing the cells made. In some cases, there is a callus stage in between, but in other cases, the embryo grows straight from a cell or tissue. The replication of the same genes present in the parent tissue facilitates somatic growth of embryos. These embryos are called clones. This method is especially helpful for breeding plants because it eliminates the need for a root face, which is needed for micropropagation using lateral buds and organogenesis. Investigations into developing somatic embryogenesis systems for legumes have been extensively studied and reviewed (Venkatachalam et al., 2003; Pratap et al., 2010). But because it is hard to grow back leguminous species in a lab, this technology isn't used as much as it could be. This makes the development of successful methods one of the most important parts of somatic embryogenesis. Recently, Ochatt and Revilla (2016) talked about some of the problems with development and suggested ways to solve them. Bobkov (2014) and Nafie et al. (2013) looked at different ways to grow cells in a lab to increase the number of spontaneous embryos that grow. During the induction phase, Bobkov (2014) used high temperatures to stress the plants and a low concentration of growth regulators. This led to calluses with structures like embryos that could be turned back into plants. On the other hand, Nafie et al. (2013) found that somatic egg production was best with MS medium that had 1.5 mg L-1 of 2, 4-D, and 0.1 mg L-1 of 24-Epibrassinolide added to it. Culture media must have the right amounts of growth regulator substances (GRS) like auxins, cytokinins, abscisic acid, and gibberellins, as well as the elements plants need to grow and develop.

Maturation results from the storage of carbs, lipids, and proteins, the drying out of the embryo, and the slowing down cellular respiration (Deo et al., 2010; Ochatt and Revilla, 2016). This process is controlled by how many different signaling pathways work together. These pathways combine genetic, biochemical, and hormonal signals. Sugars, especially sucrose, are important parts of the signaling system that starts the transition phase (Ochatt, 2015; Ochatt et al., 2010). Cabrera-Ponce et al. (2014) showed that using sucrose to lower the water potential of the growth medium is a major way to help embryos grow and develop while being grown in a lab. This method lets you make a lot of new and high-quality types in a short amount of time. However, unlike micropropagation, it is expensive and not very useful for breeding pulses. Still, it is a great method for transgenesis and causing mutations because somatic embryo culture usually starts with a single cell and doesn't allow chimaeras to form.

Organogenesis

Organogenesis is making shoots and roots from an explant (tissue) without the appearance of a callus. If, however, a callus-forming phase comes before organogenesis, this is called indirect organogenesis. This process generally starts by culturing the explant on a callus-inducing medium (CIM) high in auxin. The explant is then placed on a shoot-inducing medium (SIM) or a root-inducing medium (RIM) of a predetermined auxin-to-cytokinin ratio (Ochatt et al., 2010). Certain legumes, on the other hand, have a low rate of root production when they grow back, and they tend to lose plants during acclimatization and hardening before being put in the soil. To solve this problem, Sarker et al. (2012) made a different way for L. culinaris Medik. to grow new roots that didn't involve root growth in a lab. Also, some members of the Fabeae tribe have been able to get around this problem by using in vitro and in vivo grafts (Atif et al. 2013).

Genetic Mutation

Somaclonal variation, also called a spontaneous mutation, can happen when micropropagation and/or renewal processes are repeated. This can lead to plants that are not true-to-type. This variation can come from either the genotype or the phenotype, so it can be genetic or epigenetic. This kind of variation is usually not what you want in clonal propagation or plant transformation studies. Still, Schlichting and Wund (2014) found that it can be very helpful for breeders to find possible somaclonal types in callus-regenerated plants when they are still young. Khatun et al. (2003) say that explant types, growth regulators, genotypes, nutrient makeup, and hormone supplementation are all important factors in somaclonal variation. Elmaghrabi et al. (2013) suggested that some clones selected by either pathogens or natural forces can be beneficial. To demonstrate this, Thiagarajan et al. (2013) examined callus regeneration in various salt concentrations and found that regeneration decreased as the salt content increased. This research demonstrated the potential for selecting clones with useful traits like salt tolerance. Somaclonal variation is an interesting source of variety that breeders can use (Soniya et al., 2001).

Fusion of Somatic Cells

Conventional breeding only works for closely related species. It doesn't work for species that aren't closely related or that can't reproduce with each other. Protoplast fusion technology, on the other hand, gives a new point of view by allowing breeding between different species. This technology lets two protoplasts with different genes join to make heterokaryons, parasexual hybrid protoplasts. Symmetric somatic hybrids are composed of genetic material from two different organisms, while asymmetric somatic hybrids are composed of genetic material from two strains of the same species. Nucleo-cytoplasmic hybrids, however, comprise a nucleus derived from one parent and either the cytoplasmic genome of the other parent or a mix of the two. Ikeda et al. (2011) say that one important way these crosses can be used is to make male-sterile lines. Ochatt et al. (2005) and Ochatt et al. (2007) point out that methods for isolating and fusing protoplasts in grain legumes are not well studied, but there have been some amazing successes. For example, Durieu and Ochatt (2000) devised a plan for fusing peas (*P. sativum* L.) from different species.

Double haploid

Most of the time, the word "haploid sporophyte" refers to sporophytes with the same number of chromosomes as gametes (Palmer et al., 2005; Bhojwani et al., 2010). Double haploids (DHs) are increasingly used in plant breeding due to their homozygosity and uniformity (Germanà et al., 2011; Lulsdorf et al., 2011). By doubling the haploid complement, the number of chromosomes can be returned to normal, which is beneficial for both cultivar and hybrid crop growth. In addition, recombinant inbred lines (RILs) derived from DH lines is gaining popularity due to its applicability in identifying recessive, dominant, and harmful genes in quantitative genetics studies (Szarejko and Forster, 2007). Somatic hybridization can also be done with DH, which means that ploidy levels can be changed, and cross-incompatibility hurdles can be avoided (Germanà, 2011).

For example, wide hybridization with chromosome deletion, gynogenesis, and androgenesis (anther and microspore culture) can make haploid plants (Khush and Virmani 1996). Most people use the second one. Recently, a technology-based method called "centromere-mediated genome elimination" (Ravi and Chan 2010, Comai 2014, Tek et al. 2015) has been created to make DH in different species. Gynogenesis, also known as the in vitro generation of maternal haploids, is a way to make haploid embryos from a female gametophyte. It can be done by growing in a lab ovule, with placentas attached, ovaries, or whole flower buds that haven't been fertilized (Murovec et al., 2012). Genotype, growth conditions, microspore developmental stage, flower bud pretreatment, abiotic stress pre-treatments (e.g. centrifugation, electroporation, and osmotic shock), light intensity, photoperiod, temperature, nutrition, and season of donor plant growth have all been identified as factors influencing the efficacy of microspore culture (Germanà 2006; Hosp et al. 2007; Ribalta). Crosser et al. (2006) and Ochatt et al. (2009) demonstrated that the utilization of uni-nucleate microspores is a suitable method for initiating haploid cultures in peas (Pisum sativum). This is true for all races. In chickpeas, the best reaction came from uni-nucleate microspores (Grewal et al., 2009), especially when the buds were 2–3 mm long, and the anthers were light yellow and see-through (Panchangam et al., 2014). (Kozak et al., 2012) found that the best time for androgenesis to happen in lupin (*Lupinus angustifolius* L.) is when the buds are 5–6 mm (from the middle section of the inflorescence).

Culturing Flowers in Vitro

In vitro flowering and seed setting is a good way to speed up the creation of rare and valuable genotypes when there aren't enough seeds or to ensure that new traits stay fixed when renewed shoots are hard to root or establish. Also, the change from the vegetative to the reproductive phase can be controlled by changing physical and chemical factors and internal and external triggers. Unfortunately, growing lentils in a lab has been hard, especially when it comes to finding a good way to make roots growSarker et al. (2012) developed a method for in vitro flowering and pod formation directly from shoots regenerated in vitro by using two types of microsperma and two types of explants (cotyledonary nodes and severed embryos with one cotyledon attached). Das et al. (2012) also saw that shoots that had been changed with Agrobacterium grew three flowers in a lab and alive and healthy pods after two to three weeks. Also,

Ochatt et al. (2002) used this in vitro flowering method to speed up breeding, getting up to seven generations of peas, three generations of grass peas, and four generations of Bambara groundnuts per year. Ribalta et al. (2014) improved this protocol across a range of genotypes by using an anti-gibberellin (Flurprimidol) to reduce the length of the internodes and control plant growth. Ribalta et al. (2016) showed that early flowering and knowing the exact time of embryo physiological maturity made it easier for immature seeds to germinate, which shortened the pea's life cycle even more.

Transfer of Genes in Plants

The goal of plant transformation is to make a full plant again. This is done by delivering, integrating, and expressing foreign genes in plant cells. This is required when the desired genes aren't found in a certain species because they don't get along with each other. So far, gene delivery systems have been classified into two distinct categories: direct gene transfer and Agrobacterium-mediated gene transfer. Applying physical or chemical forces to transfer genes directly into plant protoplasts, cells, and tissues has been explored as a viable option for gene transfer. An alternative approach is agrobacterium-mediated gene transfer, which employs *A. tumefaciens* as a vector to transfer foreign genes into the host genome. The use of transgenic plants to introduce novel genotypes with beneficial traits has been widely explored (Wang et al. 2005; Sahebi et al. 2014; Ziemienowicz 2013). These genetically modified plants have been designed to resist herbicides, insect pests, and virus diseases, improve plant nutrition, and reduce the effects of dangerous agrochemicals, ultimately increasing yield components. Transgenic crops resistant to insects are the second most popular feature to be sold after transgenic herbicide resistance (James, 2013). Numerous varieties of legumes have been shown to contain transgenic plants (Atif et al. 2013). Direct gene transfer is the most effective technique for organisms, not hosts of Agrobacterium. For instance, Peas (*P. sativum* L.) are one of the most vulnerable grain legumes to salt stress. Ali et al. (2015) employed transgenic pea plants overexpressing the Na+/H+ gene from Arabidopsis thaliana to increase salt stress resistance in peas. Negawo (2015) enhanced pea insect resistance via Agrobacterium-mediated transformation. For two microsperma kinds of lentils (*L. culinaris* Medik.), a transformation mechanism was also created by Subroto et al. (2012), yielding transgenic shoots with an overall frequency of 1.009%. Bermejo et al. (2012) achieved a success rate of 7% in the production of transgenic plants. Moreover, Bermejo (2015) developed an efficient and repeatable in vitro regeneration strategy for shoot regeneration from cotyledonary node explants. Transgenesis and cis-genesis (The introduction of new genetic material into a species can be achieved by adding genes from the same species or closely related species capable of interbreeding. This can bring about genetic diversity within the population and ultimately lead to the evolution of new traits). Might speed up the breeding process by skipping many generations in the introduction of genes, yet public acceptance of transgenic plants may be unequal.

Perspectives and summary

Undoubtedly, in vitro culture methods are useful for breeding pulses. However, pulses are known to be stubborn. Its goal is to look at how in vitro tissue culture methods are used for breeding now and figure out how they could improve the quality and yield of leguminous pulse crops. The study will also examine the pros and cons of in vitro tissue culture and how it might affect breeding programs in the future. The results of this study will give us important information about how in vitro tissue culture can be used to improve the quality and output of leguminous pulse crops. It will look at how in vitro tissue culture methods are currently used for breeding and how they could be used to improve the quality and yield of leguminous pulse crops. We will talk about the pros and cons of in vitro tissue growth and what it means for future breeding programs. The study's results will give us important information about using in vitro tissue culture and how it might help improve the quality and yield of leguminous pulse crops. Pulse breeders have found that only cheap and easy-to-use methods work best for breeding programs. As a result, in vitro flowering and in vitro embryo growth are the most useful and practical methods used today. The DH method, which has been used a lot with some crops, can't be used now because no one knows how to help plants grow back. Still, it has potential and should be combined with phenomics and genetics to speed up the development of new cultivars, save money on plant breeding, and shorten the time it takes to breed pulses. To this end, more research should be done on these technologies, and the possible risks of using them should be carefully thought through before they are used on a large scale. Ducts necessitate adherence to the regulations and standards of each country to ensure their successful marketability.

Conflict of interest

The authors declared the absence of a conflict of interest.

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