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Embryo Rescue Technology: An approach for varietal development and *in vitro* germplasm conservation

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Abstract: *Embryo culture technique has been used as invaluable tool in plant breeding and in the field of in vitro culture. There are certain reasons for embryo abortion such as inability of pollen to germinate, degeneration of endosperm failure of pollen tube to grow, seed dormancy, post zygotic failure etc. Due to stagnancy in the yield of* Vigna *and paucity of the genetic variability in the primary gene pools there is a need to increase the production of quality plants and to widen gene pool of* Vigna *species.*

The gene pools can be increased by raising interspecific hybrids through embryo rescue technique. However, interspecific hybridization between Vigna *species generally fail due to different barrier to crossability and abnormalities in the pollen tube development Embryo rescue technology is the approach which can be applied to save such hybrid embryos. Progress in genetic improvement in* Vigna *species through conventional breeding methods is time consuming and labour intensive to meet with low survivability of the interspecific hybrid. Keeping in view the importance of embryo rescue technology the present study has been formulated. The study depicts that though the technique is tough but is a proven technique to raise hybrid variety that can be successfully utilized to increase the production and to widen the constricted gene pool.*

Key words: *Embryo rescue, Gene pool, Interspecific hybrid,* Vigna

INTRODUCTION

With continued to increase in demand supply of *Vigna* species in most countries in the West Asia and North Africa region there is a need for production and development of new varieties. The urban, rural peoples and farm families suffers most in these circumstances as it is the staple food which becomes beyond their reach. [Yeung EC, 1981]. There are many techniques by which new varieties can be developed. Embryo rescue technology was found to be most promising one in rasing interspecific and intergeneric hybrids. The embryo culture was begin with barley embryos [Pundhir *et al.* , 1995] and was followed by the successful rescue of embryos from nonviable seeds of a cross between *Linumperenne*"! *Linumaustriacum* [Przywara, 1989] and for full embryo development in some early-ripening species of fruit trees [Scandalios, J. G, 1969]. The difficulty in raising interspecific hybrids are basically due to sexual self incompatilbility, embryo inviability, seed dormany, haploid production etc. To overcome these problems various techniques such as Embryo culture, Ovary Culture, Ovule Culture and other techniques of controlled *in vitro* techniques has been applied to get interspecific and intergeneric hybrids (119. Raghavan, V. (1980), Zenkteler, M. (1984). Embryo rescue technology is the most promising technology. A highly efficient technique of embryo rescue has been developed by Li and his team (2014) hybrids of grape and produced disease resistant seedless cultivars.

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HISTORICAL BACKGROUND

Plant tissue culture and it's application was first proposed by Gottlieb Haberlandt, German scientist known as father of plant tissue culture. The first true plant tissue cultures were obtained by Gautheret (Gautheret, R. J. , 1934, 1935) from cambial tissue of *Acer pseudoplatanus*. After that there was series of different plant tissue culture techniques like anther culture, pollen culture, shoot tip culture, root culture and so fore. Another important technique is embryo culturethat has been used to save the hybrid products of fertilization whichmay degenerate due to various factors which will be discussed in this review article. Embryo culture is a valuable *in vitro* tool for the plant breeder often used to rescue embryos from interspecific and intergeneric crosses, seedless triploid embryos, haploids production, overcome seed dormancy, to determine seed viability and to develop plant variety from embryos that do not fully develop naturally or the embryo aborts under certain circumstances. Hannigin 1904 was the first to obtain viable plants from mature embryos of two crucifers that were isolated aseptically and grown on a medium supplemented with minerals and sugar. It was concluded that the mature embryos grew immediately, overcoming dormancy. Hu and Wang, 1986 reported many unsuccessful crosses resulted from embryo abortion. Early embryo abortion occurs due to various reasons such as the endosperm fails to develop properly or poorly developed or not developed at all. This problem may overcome by culturing the embryos in nutrient medium this helps in avoiding postzygotic barriers within the mother plant. Several successful cases have been documented with embryos arising from interspecific hybrids and intergeneric hybrids (Bridgen 1994). Yeung *et al.*, 1981 reported that embryo culture can shorten the breeding cycle by overcoming dormancy in seeds. In addition to the applied uses of embryo culture, the procedure is useful in basic studies. On other hand embryo rescue is useful when both destructive analysis of endosperm and germination of embryo are required. Li *et al.*, 2014 developed highly efficient protocol for hybrid embryo rescue from seedless *V. viniferagrapes×wild Chinese Vitis* species which resulted in a significant improvement in breeding efficiency for new disease-resistant seedless grapes.

NATIONAL AND INTERNATIONAL RATIONALE OF PULSE CROP PRODUCTION AND DEMAND

Pulses are the major plant-based protein supplement to high-nutritional food supplies which plays play a strategic role in the health and productivity of the nation. Economic development of a country is accompanied by an increase in living standards and improvement in food habits. However there is gradual reduction of dietary supplements and thus refining the nutritional requirements with increase in country's population is the matter of concern. The demand for pulses necessarily depends on the availability and prices of pulses of diverse varieties. The present market price of the pulses in India has been raised to 200 to 250 rupees/kg which is quiet high for common man of developing country. Advance farming technologies has been used to improve the problems, such as productivity and yield at the same time the demand pulse products have also to be explored to be increased for production and growth of these crops. (http://www. ipga. co. in/pulses-market-india-and-world). The pulse industry in India generally refers to a number of crops like chickpeas (locally known as chana), tur, masur, urad, **moong**, and peas. According to 2012 statistics from the United Nation's Food and Agriculture Organization (FAO), the most important pulses by production are dry beans (29. 4%), dry peas (24. 5%), chickpeas (13. 7%), dry cow peas (8. 5%), pulses (nes) (7.2%), and broad beans (5.7%). These together contributed 89% to the total global output of 70 million tonnes in 2012 (Agricultural Statistics at a Glance, 2013). India, Pakistan, Bangladesh, Sri Lanka are major consumers of pulses The market for pulses for human consumption in these countries is very high as these crops are their staple food and source of protein in the local diets. While consumption levels of pulses are increasing with the increase of the population and hence demand for production has also been increased. India despite being world's largest producer of pulses, only small exports of pulses are taking place because of restrictions on exports and the high domestic demand. The supply- demand balance sheet for pulses is provided in Table 1 which shows that India should take in India has taken initiative increasing the production as well as with development of different pulse varieties to meet the demand. India has only 15% of the area with assured irrigation facility. High level of fluctuations in pulse production and prices farmers attracted towards cash crops like Bt cotton, maize and oilseeds etc because of better return and lower risk and are not very keen on taking up pulse cultivation despite high wholesale pulse prices in recent years.

Table 1
Demand and Supply Balance Sheet for Pulses (000 tonnes)

Total pulses	*2010-11*	*2011-12*	*2012-13*	*2013-14*
Production	18,240	17,090	18,340	19,770
Imports	2,780	3,500	4,010	3,500
Total supply	21,020	20,590	22,350	23,270
Total Export	209	175	200	200
Domestic Use	20,811	20,415	22,150	23,070
Total utilization	21,020	20,590	22,350	23,270
% imports to production	15. 2	20.5	21.7	17.8

Source: (India's Pulses Scenario, 2014)

Global demand and consumption of agricultural crops for food, feed, and fuel is increasing at a rapid pace. This demand for plant materials has been expanding for many years. The world's major producers of pulses are India (23. 1%), Canada (6. 7%), China (12. 08%), Myanmar (7. 57%), and Brazil (4.03%), which together account for half of the global output (Fig. 1). In world production, dry bean is the most important going by area and production, followed by chickpea and pea. Lentil, which constitutes 4. 6% of the global pulses output, is mainly produced in India, Australia, and Canada (*www.ag.ndsu.edu*).

The United Nations, declared 2016 as "International Year of Pulses" (IYP) to heighten public awareness of the nutritional benefits of pulses as part of sustainable food production aimed at food security and nutrition. Mohanty S and Satyasai KJ (2015) provided the production data of pulse crops

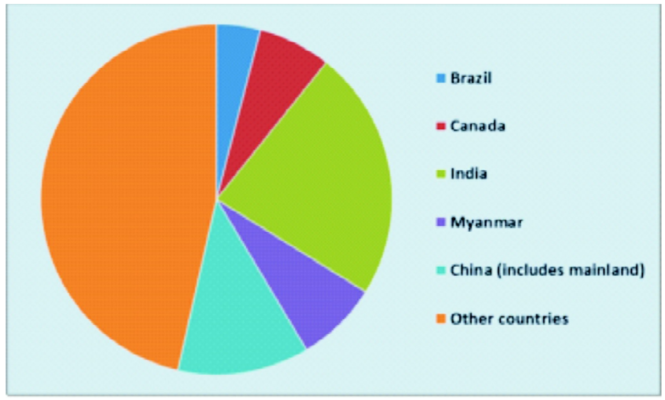

Figure 1: Share of major pulses producers in 2012

Source: http://www. ipga. co. in/pulses-market-india-and-world

according to which India is the largest producer (25% of global production), consumer (27% of world consumption) and importer (14%) of pulses in the world. Pulses are being consumed in diets across the globe andgreat potential to improve human health, conservation, protection of the environment and contribute to global food security. To meet the demand and to stabilize the farmer interest on taking up pulse cultivation, advance agriculture technologies such as embryo rescue culture and other plant tissue culture techniques should be encouraged to develop different varieties of pulse crop which can be produced throughout the year with shortened time period.

MANAGING GENE FLOW

Embryo rescue technology is applicable to produce hybrids which does not form in nature due to the possibility of embryo abortion, pre pollination barriers, sexual incompatibility however there may be chance of formation of crop weed complexes (i. e population of crops and their wild relative may exchange genes). The gene flow can be managed following different parameters such as by estimating the dispersal curves for gene flow, spatial scaling and studying the mass effectsetc. Quantitative measurements of gene flow provide critical evidence for risk assessment, containment, and monitoring procedures such as rapid qualitative assessments based on field surveys of historical behaviour in conventional crops and intensive quantitative measures based on experimental estimates and models of current gene flow.

Geneflow-Hazard Quadrant method is being usedto clarify some of the Landcare Research issues in interpreting the two components of risk of estimating gene flow exposure and identifying potential hazards. Therefore while raising utmost precaution should be taken and gene flow management should be done properly.

CAUSE OF FAILURES FOR INTERSPECIFIC HYBRIDS

Precocious germination

A major problem with embryo rescue technique is used to during the precocious germination of seed that means before germination of embryoswhich causes the formation of weak seedlings. To regulate orderly development of embryos in nature, embryos can be cultured under various conditionsto simulate embryological development. Ramming 1985 reported that the precocious germination can be prevented through ovule culture.

Malnutrition

Brink and Cooper (1947) have suggested that failure of endosperm and embryo development in interspecific hybrids is mainly due to malnutrition. The authors found out the reason of abortionwhen they the crossed Nicotianarustica x N. glutinosa. They concluded that the collapse of the hybrid seed was due to starvation of the embryo as a result of early disintegration of the endosperm, and retardation of the endosperm was associated with an overgrowth of the endothelium. The proper and balanced nutrition is required for the immature seeds to develop into mature seeds.

Cytological changes during embryogenesis

Detailed microscopical studies of the normal development and interactions of both embryo and endosperm of *Solanum nigrum* have been reported by Briggs (1993a, b, 1995, 1996) who conducted careful investigations using both light and electron microscopy. He stated that the endosperm is not passively digested by the embryo as commonly supposed. There are certain cytological changes during embryogenesis which can be controlled at early stages of can be controlled by growth regulating substances passed to the embryo from the endosperm this can be done by changing the enzyme systems and by repeated cycles of nucleic acid and protein synthesis.

Endosperm Balance Number (EBN)

Scientist has reported their findings responsible for the successful seeddevelopment. Different hypothesis were given by the scientists. Some stated that a ploidy ratio of 2:3:2 of maternal tissue: endosperm:embryo was necessary for successful seed development, some considered the endosperm:embryo ratio most important, but some favoured the maternal tissue: endosperm ratio, whereas few suggested that endosperm function was autonomous. Subsequently, various scientists have maintained the importance of the 2:1 ratio of maternal: paternal genomes in the endosperm. The Endosperm Balance Number hypothesis developed has proved useful, both in achieving complicated interspecific hybridizations involving various ploidy levels however this criterion for selection (Hawkes and Jackson, 1992). Katsiotis *et al.,* 1995 studied some inconsistencies in this hypothesis and few normal seed were obtained and hence the histology of endosperm was not considered further.

Polar-Nuclei Activation (PNA)

Polar-Nuclei Activation (PNA) hypothesis has been developed for some cereals. This hypothesis states that the endosperm develops in response to activation of the two polar nuclei by fusion with the appropriate male nucleus. Endosperm development is expressed as the 'Activation Index' which is the ratio of the 'Activating Value' of the male nucleus to the 'Response Value' of the egg nucleus or the two polar nuclei. Nishiyama and Yabuno, 1978 reported the success of this hypothesis in crossability between various species of Avena as well as Triticum and Aegilops.

Post Zygotic Barriers

Brink and Cooper, 1947, Lester and Kang, 1998 gave distinct types of post-fertilization development.

Appropriate pollinations within one species generally result in normal development of both endosperm and embryo, and production of good seeds. There are various post barriers such as embryo formation and degeneration, abortion of ovules following endosperm and embryo death, collapse of seeds during the early stages of development, crosses between diploids and their own autotetraploids generally fail due to embryo abortion. These barriers can be overcome applying embryo rescue technology.

GENERAL EMBRYO RESCUE PROCEDURES

Embryo culture

Embryo culture is one of the earliest forms of in vitro culture applied that has proven of greatest value to plant breeders majorly utilized for interspecific hybridization. Embryo culture is most commonly embryo rescue procedure, in which embryos are excised and placed directly onto culture medium. Seeds or fruits are collected from controlled pollination of greenhouse- or field grown plants prior to the time at which embryo abortion is thought to occur.

Ovule culture

Ovule culture is a technique in which the ovule itself is placed onto the culture medium when the ovary is still inside the ovule. This may prevent any damage that may cause during excision of the embryo required for embryo culture.

Ovule perforation technique

Small holes are made in each ovule just prior to its placement on the culture medium. These perforations, which should be made with care not to damage embryos, increase water and nutrient uptake. Two types of ovule support systems have been developed to culture ovules, the filter paper support system and vermiculite support system which involves culturing ovules on top of filter paper placed over liquid medium, whereas the vermiculite support technique entails placing ovules micropylar side down into a sterile vermiculite support.

Ovary culture

In ovary or pod culture, the entire ovary is placed into culture. Ovaries are collected and any remaining flower parts removed. Disinfestation protocols must remove surface contaminants without damaging the ovary. The ovary is placed into culture so that the cut end of the pedicel is in the medium. At the end of the experiment, seed are removed from the fruit that develop in culture.

Embryo-Endosperm transplant technique

This technique involves inserting the embryo from an incompatible cross into endosperm removed from a related compatible cross. For example, the embryo of an interspecific hybrid may be inserted into endosperm from an intraspecific cross involving one of the parental species. The embryo and endosperm are then placed into culture together.

Ovary Slice culture

In this technique ovaries were cut transversely into sections and the basal cut end of the sections placed on the culture medium. The ovary-slice culture procedure was considered to be the superior than ovary culture and ovule culture as it was less time consuming.

APPLICATION OF EMBRYO RESCUE TECHNOLOGY

Monoploid production

Monoploid induction and regeneration is considered as a powerful tool in plant breeding. Embryo culture and Anther culture are used to get monoploid. Monoploid obtained has advantages as they are the best source for linkage studies, useful in gene studies etc.

Overcoming seed dormancy

The other major application of embryo culture in breeding is as a means of overcoming seed dormancy. Seeds of certain species germinate very slowly or not at all under normal conditions. The cause may be in the form of endogenous inhibitors, lesser length, high temperature, storage condition and maturity of the embryo. These problems can overcome by providing specific signals for seed germination, rightly through embryo culture.

Shortening breeding cycle of plants

Embryo culture is also useful in reducing the breeding cycle of new varieties in caseswhere long dormancy causes extension of breeding cycle. thus enabling the breeder to produce two generations in one year or shortening the breeding cycle to three or four months.

Combining embryo culture and back crossing in gene transfer

The embryo culture has been proved as a viable technique for resynthesising someof the plant hybrids . Successful gene transfer has been done by back crossing the two species of different genetic composition and production of cytoplasmic sterile male plants. Thus this scheme facilitatesgene transfer overcoming the species barrier.

Production of interspecific hybrids

Wide hybridization enables the interspecific gene transfer, which may lead to the additional source of variation for the desirable characters. A possibility of getting viable seed crossing all the barriers and production of interspecific hybrids has been recorded.

DISCUSSION AND CONCLUSION

With increase in demand in pulse crop consumption, supply and production should also be increased. There is need to develop different varieties of pulse crops and increase the production to meet the demand. Plant tissue culture has been boon to the advance farmers/plant breeder to get the maximum production through out at minimum space. In this review article all the pros and cons of embryo rescue technology is explained. This study has been done to increase the genepool of pulse crop specifically with respect to *Vigna* species. It can be concluded that embryo rescue technology have huge scope to success and can be used as an aid to raise interspecific hybrids. This will play an important role in economy in countries like India where the occupation of majority of the people is agriculture. There is a greater need to make available quality seeds to the farmers in time and in sufficient quantity at reasonable prices. Applying this technology it will not only supply the quality seeds and spurious seeds but also will manage to keep up the interest of farmer to get the production in time throughout the year. This will also play major role in maintainingfood and nutritional security**.**

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