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Tissue Culture and Breeding of Maize (*Zee mays* L.) "A Review"

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Abstract: The development of pure lines of maize by the application of tissue culture is considered to be more effective to enhance high yielding hybrids development. Somaclonal variations may be more useful for genetic material exchange in wide hybrids. Disease-free superior clones can be regenerated through tissue culture techniques, which can be applied to all known infectious agents but are especially valuable in elimination viruses and viroids from vegetatively propagated crops. Producing clean planting material through *in vitro* methods can significantly raise crop yield and quality. A synthetic medium is more useful than the natural products in the medium for the callus induction and growth. As the process of regeneration may be enhanced by adding synthetic plant hormones (auxins and cytokinines) which regulate cell division and differentiation.

Key words: Anther culture, maize, breeding, somaclonal variation, embryogenesis

Introduction

Maize (*Zea mays* L.) is the most important grain crop in Pakistan and as well as other countries of the world. Maize (*Zee mays* L.) is being used as food for human being and feed for poultry. It is also grown as an important kharif fodder, alone and in mixture in the country. Maize is also an important source of products such as sweeteners, starch, alcohol, oil and feed ingredients. Due to its increasing importance to the Pakistan economy and to the problem of feeding the increasing world, it is essential that the maize gerrnplasm base be maintained and enhanced for the development of outstanding maize varieties. In view of its increasing importance, improvement of maize has picked considerable attention (Hunter, 1980; Han, 1982; Prasad and Singh, 1980; Bhole and Patil, 1983; Russell, 1985; Dai *et al.,* 1990; Hussain and Aziz, 1998; Ahsan, 1999; Mehdi and Ahsan, 1999; 2000). The dramatic increases in yields were the result of a combination of genetic improvements in hybrids and improvements in husbandry and management practices. Russell (1985) reported that nearly 60% of the improvements were the result of genetic improvements. If these 'yield improvements are to continue, the germplasm base of maize must be maintained and expanded.

Plant breeding is an art, science and technology of changing and improving the genetic architecture of crop plants that still remains the field of future for adding to nation's prosperity and well-being. This is mainly because plant breeding involve traits improvement of the crops for human economic uplift. This improvement in the plant crops can be achieved by gene reshuffling and transfer between various genomes by the use of *in vivo* method of foreign gene transfer is backcrossing (Allard, 1960; Kaul, 1988; Moreno-Gonzales and Cobero, 1993; Haywood *et al*., 1994) or *in vitro* metohod (tissue culture, genetic engineering, etc). The later techniques have been available from about last three decades but many plant breeders are still unfamiliar, Therefore, use of tissue culture for the improvement of maize crop is reviewed with the idea to provide available information in the literature to plant breeder.

Maize breeding: Maize is monocotyledonous specie and in the recent years it is also proved to be useful for biotechnological research, both on its tissue culture attributes and other economic values. Maize tissue culture technique genetic engineering is still in its infancy,. as regeneration of maize plant from callus culture for the first time was reported by Green and Phillips (1975). About 15 years after this initial research, a fertile transgenic maize plant was introduced (Fromm *et al*., 1990). The development of pure lines of maize by the application of tissue culture is considered to be more effective, therefore anther culture of maize got attention of breeders throughout the world (Straus, 1954; Straus and LaRue, 1954; Shu-Hua *et al*., 1978; Fromm *et al*., 1990). However for outstanding improvement in maize crop production to feed the increasing population of the world, it is necessary to use plant biotechnological approaches in maize breeding. The original landrace varieties of maize germplasm represented a wide range from the tali, late, white southern dents concentrated in the southeast arid the early flints located 42 to 45 degrees north latitude. Brown and Goodman (Brown and Goodman, 1977) assigned the pre-hybrid U.S. maize germplasm to nine broad racial complexes. Shull (1909) suggested the pure line-hybrid concept, breeding efforts were focused on testing the concept to provide a practical method for developing lines for use in hybrids. Shull's concept was extensively tested and developed to the point where it is considered one of plant breeding's greatest achievements. By the 1930s, double-cross hybrids were available for farmer use. Hybrids were rapidly accepted.

Inbred lines developed for use in the original hybrids were derived from the open-pollinated varieties. This approach was a natural one because the landrace varieties were the primary source of adapted, improved germplasm. Subsequently, pedigree selection was initiated in segregating populations derived from inbred line crosses and the relative importance of the landraces as breeding gerrnplasm decreased rapidly (Jenkins, 1978), Breeding methods to exploit the pure line-hybrid concept of maize breeding reduced the interest and emphasis given to the original landrace varieties.

After the initial pause in hybrid improvement, development of successive generations of recycled inbred lines resulted in continuing increases in yield and general performance of hybrids. Interest in the genetic causes of improved performance and the hope for continued improvement stimulated quantitative genetic and selection studies within

Table 1: Composition of the media for callus induction from maize plant (*Zea mays* L.) cv. Surprise Sweet, cv. Black Mexican Sweet (Straus and LaRue, 1954)

Plant part	Medium	Natural nutrients	Culture conditions
Endosperm	White (major element) Nitsch (minor element)	Yeast extract	Agar

and between maize populations (Gowen, 1950). Most of the evidence suggested that the genetic variation was primarily due to additive genetic effects with partial to complete dominance of the alleles at segregating loci. There were some exceptions: with F_2 populations derived from inbred lines, over dominance was detected but this was found to be due to repulsion phase linkage bias and significant non-additive effects were detected from analyses of generation means and types of hybrids. Generally, it seems that additive genetic effects are of greater importance and breeding and selection schemes that effectively capitalize on selection method. Studies demonstrated that sufficient genetic variability was present in maize germplasm to allow continued genetic progress (Hallauer and Filho, 1981). Texas male sterile cytoplasm (Toms) was found to be a very efficient method for the production of hybrid seed without detasseling. Other cytoplasmic male sterile systems were available and had been used but the Tcms system was more broadly useful and reliable than the others (Duvick, 1965).

Adequate and useful genetic variation is of concern to all involved with maize germplasm, breeding and production. The issues of genetic vulnerability have been discussed and inventories made of the materials used and available. Because of the nature of maize germplasm, breeding methods used, cultivars available for productibn and the level of research activity conducted in maize, genetic variation is available to minimize risks of a widespread catastrophe. However, sophisticated new technologies can be used to estimate the amount of genetic diversity among maize hybrids (Smith and Smith, 1992). Isozymes, protein characterization and molecular markers can be used to detect the degree of relatedness of maize hybrids, even without knowledge of their pedigrees. Germplasm sources are an important component of the germplasm included in breeding programs, ranging from F_2 populations derived from crosses of elite inbred lines to genetically broad-based composites. The types of germplasm included depend on the major objectives of the breeding programs. Duvick (1981a, b) summarized the status of genetic resources and urged that maize breeders develop and put into practice precise methods to increase useful genetic diversity in maize (Table 1).

Anther culture: Anther culture is applied to obtain homozygous plants in a short time. In this method, immature anthers from differentiating population (F_2) are grown on substrate. Anther culture is usually carried out at beginning of a breeding programme. Once a new population of homozygous plants is available, new varieties are developed by mass selection and pedigree selection.

Cell and tissue culture technique has been widely used to study the growth, metabolism etc. of dicotyledonous plants (Yuan-Irng and King-In, 1978; Cheng *et al*., 1980; Facciotti *et al*., 1985; Barwale and Widholm, 1987; Christou *et al*., 1987; Famelaer *et al*., 1990; Dhir *et al*., 1991a and b). However tissue culture studies with monocotyledonous plants are relatively few because of the following reasons. (a) Little success in inducing callus and maintaining better growth (LaRue, 1949; Straus and LaRue, 1954; Norstog, 1956; Tamaoki and Ullstrup, 1958). (b) Difficult to obtain a suitable size of homogenous tissue from momocotyledonous plants. The complete success in induction of callus and better growth was gained by the appliaction of auxins in the culture media used for cereals tissue culture (Yamada *et al*., 1967). At least in maize a success was gained in callus induction by the application of only yeast extract (Straus, 1954; Straus and LaRue, 1954), Tissue culture methods are used by nearly 600 companies throughout the world to produce more than 500 million plants annually for almost 50,000 varieties of plants. Currently, almost all of these plants are produced by micropropagation. There are two principal pathways for *in vitro* plant regeneration. (1) The most common is micropropagation. It involves the suppression of apical dominance resulting in the activation and multiplication of axillary buds. (2) In somatic embryogenesis cotyledon containing embryos with a root-shoot axis are formed from somatic cells. At the present time there is a much better understanding of the biology of micropropagtion than of somatic embryogenesis (Levin *et al*., 1988; Akita and Takayama, 1994; Akita *et al*., 1994; Vasil, 1994). Somatic embyogenesis is an adventitious propagation. The advantages of this method compared to other micropropagation methods are the far greater propagation factor and much reduced labor intensiveness. There are two options, once an appropriate number of somatic embryos has been obtained. (1) Somatic embryos may be placed on a substrate and grown to mature plants. (2) Somatic embryos can be dried and coated, so that they resemble seed. They can now grown the field like seeds. Slow growth techniques for medium-term storage allows clonal plant material to be stored under tissue culture conditions with extended subculture intervals. Cryopreservation techniques may be applied for the longterm storage of plant material such as zygotic or somatic embryos, shoot tips and pollen (Ashmore, 1997). *In vitro* cultures are free from exogenous fungal and bacterial contaminants and may be virus-free, if produced from meristem-tip culture. This offers advantages for the production and supply of healthy plants for distribution and use when compared with field-propagated or field-collected material (Haywood *et al*., 1994). The meristem and shoot tip explants are easier to establish if obtained from shoots in their active vegetative growth stage. For example, shoot tip explants of dwarf apple were established readily when obtained from newly grafted shoot rather than taken directly from orchard trees. In addition to physiological age of the plant, the growing environment of donor plants also exerts tremendous influence on establishment (Vasil and Thorpe, 1994). In the development of potato cultivars, until true potato seed problems are resolved, disease-free seed tubers could be supplied by culturing potatoes under sterile laboratory conditions. Methods are available to grow plants to maturity from shoot-tip explants comprising the apical meristem and at least one primordial leaf. This explant

material is not necessarily pathogen free, so *in vitro* culture techniques should include:

1. Identifying and eliminating diseased organisms
2. Testing regenerated plants for freedom from pathogens
3. Propagating healthy plants under conditions that prevent reinfection
4. Medium and long-term storage of germplasm and its distribution

In vitro propagation techniques offer the possibility of rapid clonal multiplication of disease-free plants, in a sterile, controlled environment, independent of season and with very limited space requirements. Such micropropagation may particularly be useful when species are rare or endangered. Classical propagation techniques require juvenile material which may not be available (e.g. tree species), natural regeneration processes require several month or years (e.g. tree species), or large quantities of plant material are required for distribution and use. Micropropagation techniques are now available for a large number of plant species and in many cases the propagation of plants using tissue culture is on a commercial scale. Examples of the commercial production of planting material are strawberry, potato and Musa ssp. (Ashmore, 1997). Spontaneous variation, which is exhibited by cultured plant cells may be transmitted in sexual crosses (Meins, 1983) and this somaclonal variation is recognized, as it is related to species and rout of regeneration. Somaclonal variations may be useful in exchanging of genetic material for the introgression of alien genes in wide hybrids (Larkin and Scowcroft, 1981).

Haploid can be produced from all crop species by the use of tissue culture techniques with low cost and that haploid can be exploited in any breeding programme (Jensen, 1983). The haploid cells are able to develop a haploid embryo or callus *in vitro.* Attempts to culture unfertilised ovules or ovaries have been made with many plant species but, in most cases, growth stopped at the callus stage. The method is, however, of particular importance in those crops. where anther culture is unsuccessful or problematic. The procedure for parthenogenesis is generally quite similar to the classical methods of culturing other plant tissues. Donor genotype is thought to play a decisive role (Keller, 1990). As far as the, developmental stage of the parthenogenetic cells is concerned, it is accepted that a broad range of growth stages is suitable for growth induction (Cappadocia *et al*., 1988). Regeneration rates are quite low, usually only one or a few embryos develop from an embryo sac. Despite successful *in vitro* fertilization, an embryo may not develop or be rejected prematurely. There are several reasons for this. The endosperm might be underdeveloped or the embryo and the endosperm may somehow be mismatched. The purpose of ovary and embryo culture is to transfer the embryo to an artificial nutritive substrate at an early stage, so that it need no longer depend on the endosperm. In ovary (plaque) culture, the entire ovary or slices of the ovary (plaques) are placed on the substrate.

Mode of pollen development: There are two modes of development in maize anther culture from the dividing of the cell to differentiate into pollen plant, i.e., embryonic pollen and callusing pollen. There is no definite distinction between these two modes of development except that embryonic pollen is uninucleate, undergoes repeated divisions to form multicellular embryoid, whereas callusing pollen undergoes a few divisions to form callus which differentiated into plantlets. Shu-Hua *et al*. (1978) reported in maize that the medium supplemented with casein hydrolysate is favorable to the formation of embryoid in maize anther culture. This may be due to rich amino acids contained in casein hydrolysate. However, Rijven (1956) reported that glutamine has obvious effects on the growth of young embryos. Sunderland (1971) reported in detailed review that there are four modes of pollen development in maize anther culture: (al development by way of vegetative cell, (b) development by way of reproductive cell, (c development by way of both vegetative and reproductive cells and (d) Development by the equal division of uninucleate.

Media for maize callus induction: The main media used for maize callus induction (Straus and LaRue, 1954) is given' in Table). Natural products in the media should be avoided to use, while performing experiment with tissue because they contain unknown factors (Straus, 1954). Therefore concrete efforts were made towards synthetic medium development (Straus, 1960). Schenk and Hildebrandt (1972) used a synthetic medium (Table 2) for the induction and growth of monocotyledonous plants.

Table 2: Composition of SH medium for callus induction from maize *Zea mays* L.) plant (Schenk and Hildebrandt, 1972)

	Components SH medium
Major element	
KNO_3	2500.0 mg/l
$MgSO_4.7H_2O$	400.0 mg/l
$NH_4H_2PO_4$	300.0 mg/l
$CaCl_2.2H_2O$	200.0 mg/l
Minor element	
$MnSO_4.H_2O$	10.0 mg/l
H_3BO_3	5.0 mg/l
$ZnSO_4.7H_2O$	1.0 mg/l
KI	1.0 mg/l
$CuSO_4.2H_2O$	0.2 mg/l
$NaMoO_42H_2O$	0.1 mg/l
$CoCl_2.6H_2O$	0.1 mg/l
Iron compounds	
$FeSO_4.7H_2O$	15.0 mg/l
Na_2EDTA	20.0 mg/l
Organic	
Inositol	1000.0 mg/l
Thiamine Hcl	5.0 mg/l
Nicotinic acid	5.0 mg/l
Pyridoxine Hcl	0.5 mg/l
Growth-regulating substances	
2,4-D	0.5 mg/l
CPA	2.0 mg/l
Kinetine	0.1 mg/l
Sucrose	30.0 g/l
Agar	6.0 g/l

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