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ROLE OF GENEBANK IN MAIZE (*ZEA MAYS* L.) AND WHEAT (*TRITICUM AESTIVUM* L.) BREEDING

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ABSTRACT

Maize breeding In the last three decades, a large number of maize hybrids have been developed from genotypes with a restricted genetic base. In order to decrease the genetic vulnerability, it is very important to widen the genetic base for maize breeding by the application of various methods. One particular way to increase genetic variability is treatment with various mutagens. After more than twenty-five years of research, it has been proved that such lines can be produced by mutation. In 1995, a maize gene bank was established with 1,500 lines in our department. There is large genetic variation in the maize gene bank, the exploitation of which is only possible using suitable methods of selection and evaluation. As a result of mutation, the new maize inbred lines P26, P61 and P62 were released after DUS tests. Radiation generated conspicuous changes in the plant characteristics. The most pronounced aberrations were observed for the expression of anthocyanin coloration and flowering time in various plant organs.

Wheat breeding The study was designed to examine the effects of four sucrose concentrations (45, 60, 75 and 90 gL^{-1}) and four maltose concentrations (65, 100, 135, 170 gL^{-1}) on callus induction, plant regeneration and green plant proportions. Anthers of Pavon 76 were cultured on these induction media and embryoids induced were transferred to the standard regeneration medium to compare for differences in green plant percentage and the influence of carbon sources on it. The green plant ratio showed a linear correlation with the concentration, being the highest (22.3 green plants/100 cultured calli) at 90 gL^{-1} fructose and that (31,7%) at 170 gL^{-1} maltose.

Keywords: genetic diversity, maize (*Zea mays* L.), mutation breeding, winter wheat, plant regeneration

INTRODUCTION

Maize breeding Since the 1920s, the hybrid maize production in the world has been based on the development and crossing of inbred lines. Currently in maize, many new hybrids have been developed from crosses of a limited number of parent lines. This represents a great risk for the loss of genetic diversity in elite germplasm (HALLAUER ET AL., 1988; cit. MESSMER ET AL., 1993). For these reasons and others, maize breeders have a keen interest in the characterization of genetic diversity among parental lines. The genetic diversity among breeding materials could help to prevent the great risk of increasing uniformity in the germplasm and could also ensure long-term selection gains. Due to specific breeding aims and gene erosion, the genetic basis of maize breeding has decreased significantly in Hungary in recent decades, with well-known unfavourable effects (TÓTH AND PEPÓ, 2003). To select maize varieties with more favourable genetic structures, it is necessary to enlarge the basic genetic materials and enrich the available gene sources (PÁSZTOR, 1992). The success of plant breeding depends mainly on the genetic diversity of the basic material. Crossing and mutation are different methods which are applied to generate genetic diversity (HAJÓS NOVÁK ET AL., 1996). According to RADY AND NAGY (1996), in the interests of greater yield stability, the aim is for each maturity group in each growing area to be represented by hybrids of different genetic origins. When creating hybrids, lines with diverse genetic origin should be used in order to achieve a greater heterosis effect (RADY AND NAGY, 1996).

In recent years, maize hybrids have been highly productive, but their reduced genetic variability leave them with a reduced capacity to deal with new diseases and pests (e.g. as the European corn borer in the 1940s and 1950s or southern corn leaf blight in 1970 in the U.S. Corn Belt (DUVICK AND CASSMAN, 1999), and other changes in environmental conditions. Sustainable agriculture is not new in maize breeding, most of the modern hybrids can utilize fewer external inputs (e.g. pesticides, fertilizers) by high productivity, and caused minimal environmental impacts. However with the integration of *in vivo* and *in vitro* techniques in maize breeding programs, we can obtain desirable agronomic attributes, accelerate the breeding process and enhance the genes responsible for them (PEPO, 2004).
The aim of this study was to reveal genetic variability in inbred maize lines (P26, P61, P62) produced by physical mutagens (fast neutron irradation) on the basis of morphological characteristics (expression of anthocyanin coloration, flowering time).

Wheat breeding In wheat, the low level of callus induction from microspores and subsequent plant regeneration, and high percentages of albino plants *in vitro* limited the application of haploids in plant breeding and genetic research for cereal crops (QUYANG, 1986). The yield of green haploid plants in anther culture depends on three independent components: embryoid production from cultured anthers, plant regeneration from the embryoids and the percentage of green plants (SZAKACS ET AL., 1988). Researchers have therefore shifted their efforts to investigating the influence of medium components on the proportion of green plants produced (BJORNSTADT ET AL., 1989; ZHOU, 1990). Sucrose as osmotic agent not only acts as a common source of carbon in the cell culture media of cereal (AL-KHAYRI AND AL-BAHRANY, 2002) and energy but also as an osmoticum during organogenesis (HUANG AND LIU, 2002) and accumulated in many plant tissues in response to environmental stress, including water deficit (RAMOS ET AL., 1999) for playing a role in osmoregulation and cryoprotection. It has also been reported that sucrose in lower concentration (2% and 4%), is necessary for optimal growth and multiplication (HAZARIKA, 2003).

MATERIAL AND METHOD

Maize breeding

The story and establishment of a maize gene bank by mutation in Debrecen

In Debrecen, mutation breeding was initiated in 1960 by Károly Pásztor. Within the programme started in 1979-80, F1 maize hybrid seeds were treated by radiation of Co60 isotope. Later, in 1985 and in 1991, the trial was expanded and the seeds were treated by radiation of fast neutrons at the Atomic Research Institute of the Hungarian Academy of Sciences in Debrecen. The selected mutant lines - developed in this way - have been self - pollinated for several years. In 1995, a maize gene bank was established with 1,500 lines, which are registered by the IBPGR (FRISON AND SERWINSKI, 1995). The experiment was set in the demonstration garden of the Department of Genetics and Plant Breeding in the Agricultural Centre, at the University of Debrecen.

Importance of induced mutagenesis today

Induced mutation has become an effective tool for plant improvement and has also increased bio-diversity. According to the FAO/IAEA data in 2001, the quantity of new plant varieties selected by mutation breeding has been increasing from year to year. As of June 2000, 2,252 new mutant varieties have been released in 50 countries all over the world. These new plant varieties originated from 154 plant species, and 1,275 of the 2,252

new mutant plants are agricultural plant varieties, mostly cereals (rice, barley, wheat and maize). In reality, it would be expected that the number of released varieties is much higher than listed, as many mutated genes have been used in cross breeding programmes without indicating the nature of the desired genes (MALUSZYNSKI ET AL., 2001).

Wheat breeding

A spring wheat cultivar, 'Pavon 76' was used as donor material. Liquid potato 4 (P4) medium (OUYANG, 1986) was used as a standard induction medium. Spikes were selected at random from a bulk from which 100 anthers were excised for each petri dish, with four replicates per treatment. The callus induction frequency was the number of calli obtained from 100 anthers plated 40 d following induction. Embryoids induced from the anthers were transferred to a '190-2' medium for plant regeneration when they reached 1 mm in diameter. The standard regeneration medium contained 30 gL^{-1} sucrose and was solidified using 6 gL^{-1} agar. No plant growth regulator (PGR) was included in the regeneration medium. The time of transfer was based on the size of the calli that developed between 30 to 40 d after anther plating. After 30 d of regeneration culture the number of calli that developed into albino or green plants was recorded. Plant regeneration frequency was the number of calli producing plantlets per 100 calli transferred. The green plant regeneration frequency was the number of calli producing green plantlets divided by the number of total calli producing either green or albino plants.

RESULTS

Maize breeding

Heterosis breeding in maize has caused gene erosion by using uniform inbred lines (PEPÓ AND TÓTH, 2004). In order to avoid this, an *in vivo* maize gene bank has been established which contains inbred lines with greater genetic potential and better ecological adaptation. After more than twenty-five years of research, it has been proved that such lines can be produced by mutation. The most important data of the gene bank are shown in *Table 1.*

Table 1. Number of inbred lines in the maize gene bank produced by mutation

Lines	Years									
	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
Characterization*	155	155	155	155	155	155	122	106	92	90
Maintenance**	517	450	450	450	217	217	250	310	120	102

*: According to UPOV
**:By self-pollination

Table 2. Pedigree of registered inbred lines

Lines	Hybrids	Type of irradation	Dose [Gy]
P26	**F_1(Pi 3747 SC) M_2**	fn	7.5
P61	**F_1(Pi 3901 SC) M_2**	fn	12.5
P62	**F_1(Pi 3901 SC) M_3**	fn	7.5

F_1 : First generation after crosses
M_n : n^{th} mutational generation
fn : Fast neutrons (produced in a cyclotron)

There is large genetic variation in the germplasm utilised, the exploitation of which is only possible using suitable methods of selection and evaluation. In 1985, we applied a physical mutant agent (fast neutron) for irradiation of F_1 hybrid seeds by 7.5-12.5 Gy dose. After selection, mutant lines were used for self-pollination over many years. As a result of this

selection process, P26, P61 and P62 lines have been governmentally released by the OMMI (2001). These lines serve as a basic material in our future breeding programs. Identification and the origin of these genotypes are given in *Table 2.*

As a result of mutagenic treatment, morphologically very different mutant populations were obtained. Radiation generated conspicuous changes in the plant characteristics described by UPOV in comparison with the basic material (*Table 3*).

Table 3. Comparison of characteristics (UPOV TG/2/6) of basic hybrids and the mutant registered lines (P26, P61, P62) derived from them

Basic hybrids, irradiated lines		Characteristics Code denoting degree of expression of the characteristics				
		Intensity of anthocyanin coloration of silks	Anthocyanin coloration of anthers	Anthocyanin coloration of glumes excluding base	Anthocyanin coloration at base of glume	Flowering time
Initial stock	F_1(Pi 3747 SC) M_2	5	7	7	1	72
Irradiated stock (fn 7.5 Gy)	P26	1	1	1	1	69
Initial stock	F_1(Pi 3901 SC) M_2	5	9	9	1	71
Irradiated stock (fn 12.5 Gy)	P61	1	5	5	1	68
Initial stock	F_1(Pi 3901 SC) M_3	7	7	7	9	70
Irradiated stock (fn 7.5 Gy)	P62	1	5	5	1	68

The most pronounced aberrations were observed for the expression of anthocyanin coloration in various plant organs. The variability manifested in the changes of pollination interval. Flowering time is considered to be quantitatively inherited, and different studies have identified loci that affect this trait in maize (BEAVES ET AL., 1991; cit. OLIVEIRA ET AL., 2004). Mutation treatments (fast neutron) induced earliness in flowering time of different inbred lines. Using these lines as crossing parents, they could cause earliness in hybrids. With earlier flowering time, we can avoid frequent drought periods, which reduce fertilization in maize. Earlier maturity could reduce grain moisture in harvest time, drying energy and fungi diseases (e.g. *Fusarium* ear rot). These characteristics are suitable for sustainable agriculture. We concluded that the cyclotron can be successfully applied in widening genetic variability. We produced a number of inbred lines with wide genetic variability using mutation breeding. We can use this information to develop maize hybrids, which can be useful in our breeding program.

Wheat breeding

The callus induction response to sucrose for v. 'Pavon 76' was higher at all sucrose levels than that of maltose (*Table 1*).

Table 1. *In vitro* green plant proportion in cv 'Pavon 76'

Induction medium g L^{-1}	% of green plants (green plant/No of embryoids)*	Plantlet No/100 calli transferred*	Green plant No/100 calli transferred*
Sucrose 45	22.3 a	50.4 a	11.2 a
Sucrose 60	29.5 b	50.7 a	15.0 b
Sucrose 75	37.3 c	50.8 a	20.6 c
Sucrose 90	42.9 c	51.4 a	22.3 c
Maltose 65	25.0 a	40.9 a	10.2 a
Maltose 100	39.1 b	45.8 b	20.3 b
Maltose 135	64.5 c	47.7 b	30.8 c
Maltose 170	66.0 c	48.0 b	31.7 c

*Values followed by the same letter are not significantly different according to the protected LSD test at P = 0,05.

Pavon 76 showed basically linear responses to sucrose and maltose concentrations. Some trends emerged from the response for callus induction across increasing sucrose and maltose concentrations: (i) the callus induction increased dramatically for both induction media when either sucrose or maltose were increased from 45 to 90 gL^{-1} and from 65 to 170 gL^{-1}, respectively, (ii) callus induction from Pavon 76 can be increased at sucrose levels > 90 gL^{-1} and at maltose levels > 135 gL^{-1} significantly in the induction medium. 66.0% embryoids from the 170 gL^{-1} and 64.5% embryoids from the 135 gL^{-1} maltose medium produced green plants, whereas only 42.9% embryoids from the 90 gL^{-1} sucrose medium produced green plants (*Table 1*). Sugar has two functions in culture media, as a carbon source and as an osmotic regulator. Since the sugar content of media was found to change little during the culture period, the major function of sugar may be as regulator of medium osmolarity. Thus, the difference in green plant percentages between maltose concentrations demonstrates the effect of medium osmolality on albinism. Sucrose in induction media is rapidly hydrolyzed to fructose and glucose, increasing the medium osmolarity, whereas no detectable osmotic change occurs in maltose containing medium.
A distinct response pattern types were found for plant regeneration across increasing concentration in the induction medium; the plant regeneration for 100 calli transferred was 50.4-51.4% in medium containing various amount of sucrose and 40.9-48.0% for that with maltose (*Table 1*). However, the green plant proportion showed a linear response with concentration and was the highest one for sucrose (22.3 green plant number/100 calli transferred) at 90 gL^{-1}, and that for maltose (31.7%) at 170 gL^{-1}, which were not significantly different from 75 gL^{-1} sucrose and 135 gL^{-1} maltose concentration, respectively.

CONCLUSIONS

Maize breeding

In 1995, a maize gene bank was established with 1,500 lines in our department. There is large genetic variation in the maize gene bank, the exploitation of which is only possible using suitable methods of selection and evaluation. As a result of mutation, the new maize inbred lines P26, P61 and P62 were released after DUS tests. Radiation generated conspicuous changes in the plant characteristics. The most pronounced aberrations were observed for the expression of anthocyanin coloration and flowering time in various plant organs.

Wheat breeding

Results from this study have profound implications on the choice of carbon sources and concentrations. Many of the reported effects of medium modifications and pretreatments may be related to osmotic potential. In addition, if the hypothesis about the importance of medium osmotic potential is correct, more attention should also be paid to establishing the optimal osmotic potential for regeneration media. Currently, most researchers use 90 gL^{-1} sucrose in incubation media, but only 30 gL^{-1} sucrose in regeneration media. Because of the hydrolysis of sucrose in induction media, difference in osmotic potentials between the induction and regeneration media during the transition phase of culture is much greater than expected. This difference also may have a significant impact on green plant percentage.

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