Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9275
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v35i1.15545

Immature tassels as alternative explants in somatic embryogenesis and plant regeneration in south Brazilian maize genotypes

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ABSTRACT. Somatic embryogenesis and *in vitro* plant regeneration are fundamental processes in the obtainment of transgenic maize plants. Explant, genotype and culture medium are determining factors in these processes. Immature embryo explants and the American Hi-II genotype have been widely employed to acquire genetically modified plants in this species. However, the use of more readily available explants is desired as well as the development of genetic transformation protocols for productive genotypes adapted to local conditions. This study provides an evaluation of immature tassel explants in relation to embryogenic callus production and plant regeneration in South Brazilian maize genotypes for their use in genetic transformation experiments. Immature tassels from 5 hybrids were cultivated in different callus-induction media. The frequency and the fresh mass of embryogenic calli were evaluated. The frequency was influenced by genotype, and the fresh mass was influenced by genotype and culture medium. In plant regeneration, shoots, complete seedlings and acclimatized and fertile plants were quantified. Treatments producing long term embryogenic calli from immature tassels of South Brazilian genotypes with the capacity to regenerate were identified.

Keywords: embryogenic callus, tissue culture, explants, *Zea mays*.

Pendão imaturo como explante alternativo na embriogênese somática e regeneração de plantas em genótipos sul brasileiros de milho

RESUMO. A embriogênese somática e regeneração de plantas *in vitro* são processos indispensáveis para obtenção de plantas transgênicas de milho. Explante, genótipo e meios de cultura são fatores determinantes destes processos. O explante embrião imaturo e o genótipo americano Hi-II tem sido amplamente empregados para a obtenção de plantas geneticamente modificadas nesta espécie. No entanto, é desejável a utilização de explantes mais prontamente disponíveis, bem como o desenvolvimento de protocolos de transformação genética para genótipos produtivos e adaptados às condições locais. Objetivou-se neste trabalho avaliar o explante pendão imaturo quanto à produção de calos embriogênicos e regeneração de plantas em genótipos sulbrasileiros de milho para o uso em experimentos de transformação genética. Pendões imaturos de cinco híbridos foram cultivados em diferentes meios de indução de calos, sendo avaliada a freqüência e massa fresca dos calos embriogênicos. A freqüência dos calos embriogênicos foi influenciada pelo genótipo e a massa fresca foi influenciada pelo genótipo e meio de cultura. Na regeneração de plantas foi contabilizado o número de brotos, plântulas completas e plantas aclimatizadas e férteis. Foi possível identificar tratamentos que produziram calos embriogênicos de longa duração com capacidade de regeneração de plantas a partir de pendão imaturo de genótipos sulbrasileiros.

Palavras-chave: calos embriogênicos, cultura de tecidos, explantes, *Zea mays*.

Introduction

Maize is a liliopsida of great agricultural importance for the South Region of Brazil, and it is increasingly requested due to the expansion of activities associated with industrialization and animal exploitation.

Products molecularly improved from this species are already being cultivated in Brazilian farms. In addition, genetic engineering has permitted the introduction of genes into maize that improve its nutritional value (CARNEIRO et al., 2000; GRANDO et al., 2005; NAQVIA et al., 2009; ZHU et al., 2007) and tolerance to important abiotic stresses due to drought or cold temperature (AL-ABED et al., 2007; SHOU et al., 2004; ZHANG et al., 2005).

The first stage in the obtainment of transgenic plants involves the development of tissue culture

protocols because the gene is introduced at the level of topipotent cells, and the plants can be regenerated from these cells. Indirect somatic embryogenesis has been the morphogenetic process of preference in the regeneration of transgenic plants (FRAME et al., 2002, 2006, 2011; HUANG; WEI, 2005; ISHIDA et al., 2007; VEGA et al., 2008; ZHAO et al., 2001).

Nevertheless, maize is considered a recalcitrant species *in vitro*; therefore, responses have only been obtained through the use of very young explants containing embryogenic or meristematic cells (SHARMA et al., 2005). Highly competent explants produce long term embryogenic calli that survive through a long selection period, an obligatory stage during the process of genetic transformation, and maintain their plant regeneration potential. Plant regeneration from these calluses occurs via germination of somatic embryos with well-defined root/shoot axes (ARMSTRONG; GREEN, 1985). Plant regeneration using maize tissue culture was first reported by Green and Phillips (1975) who used immature embryos as explants. Plants regenerated from somatic embryos present higher genetic integrity when compared with plants regenerated through the process of organogenesis.

The success of regeneration protocols is subject to limitations associated with the genotype, somaclonal variation, low frequencies of callus induction and the very process of *in vitro* plant regeneration (TOMES; SMITH, 1985). Generally, these factors limit the effective production of transgenic plants (SAIRAM et al., 2003).

The first stage of plant regeneration from maize somatic embryos is related to somatic embryo maturation and shoot induction development via cultivation in a culture medium with a high sucrose concentration (60 g L^{-1}), and this regeneration initially occurs in the absence of light and without the addition of growth regulators. The second stage of the *in vitro* plant regeneration of maize is related to the development of shoots and roots, which are more frequently obtained through a reduction of the sucrose concentration (FRAME et al., 2002, 2006; LEE et al., 2007; VEGA et al., 2008).

Two types of embryogenic calli have been described in maize culture. Type I is hard, compact, nodular, yellow-white colored and may proliferate as a mixture of complex tissues showing somatic embryos (ARMSTRONG; GREEN, 1985). Furthermore, these authors characterized these Type I calli by slow growth or difficulty of being cultivated throughout a long period of time. Type II calli have been observed in few maize genotypes and are characterized as being highly friable, soft and fast at growth. In addition, they have a high embryogenic activity rate, which permits its cultivation for a long period of time.

Embryogenic callus induction is dependent on various factors such as culture medium, genotype and the explant type employed to start the *in vitro* process. In relation to the composition of the medium for callus induction, the most widely used nutritious media for embryogenic callus induction in grasses is the MS basic medium (MURASHIGE; SKOOG, 1962). This medium has been used for the production of genetically modified plants of specific lines by Frame et al. (2006, 2011). Alternatively, N6 basic medium (CHU et al., 1975) has been successfully employed in the induction of the type II friable callus in model genotypes such as A188 and B73, the Hi-II hybrid and other genotypes (ZHAO et al., 2001; FRAME et al., 2002, 2011; HUANG; WEI, 2005; ISHIDA et al., 2007; VEGA et al., 2008). Gorji et al. (2011) observed that the N6 medium, which contains lower nitrogen levels than the MS medium, promoted a higher induction and maintenance of embryogenic calluses in elite maize lines.

The most widely used synthetic auxin for embryogenic callus induction in grasses is 2,4-D (2,4-Dichlorophenoxyacetic Acid). However, the addition of citokynin in the presence of 2,4-D may increase the embryogenic response in some species of grasses (GRANDO et al., 2002).

Genotype is another factor that has a fundamental role during embryogenesis induction in maize (FRAME et al., 2006; HUANG; WEI, 2005; ISHIDA et al., 2007; LEE et al., 2007, WANG et al., 2007). The embryogenic potential of Brazilian maize genotypes was reported by Santos-Serejo and Aguiar-Perecin (2000), Carneiro et al. (2000), Petrillo et al. (2008) and Fernandes et al. (2008). However, no reports exist regarding transgenic plants obtained from South Brazilian maize genotypes.

The most widely used explant for establishing the embryogenic callus culture for genetic transformation in maize is the immature zygotic embryo (FRAME et al., 2002, 2006; HUANG; WEI, 2005; ISHIDA et al., 2007; VEGA et al., 2008). However, the production of immature embryos is a demanding process, which requires time and laborious activities, such as planting, pollination, excision of the immature embryo, and dependence on plant growth in the greenhouse. These explants are seasonally available, and a narrow time range exists for the explant to be in its best condition for in vitro cultivation (FRAME et al., 2011; GORJI et al., 2011). The use of more readily available explants is one of the greatest

challenges for rendering the genetic transformation process faster and more effective (CHENG et al., 2004). Therefore, the use of explants that do not rely on the immature embryo aims at accelerating the process of obtaining in vitro plants.

In maize, the immature tassel was first used by Songstad et al. (1992) in a cultivation system based on N6 medium supplemented with 2,4-D, L-proline and $AgNO_3$ for the obtainment of type II embryogenic calli in A188 and B73 model lines as well as its Hi-II hybrid. The advantage of using the immature tassel is its rapid obtainment of plants within 6-7 weeks, with no need for growing them until the reproductive stage, pollination, fertilization, or development of the immature embryo. Primordia of young and non-emerged flowers with pre-meiotic flower buds are considered excellent explants for starting embryogenic cultures in grasses. In these explants, embryogenic calluses are primarily formed through the proliferation of the young floral primordium.

To accelerate the obtainment process of *in vitro* plants for genetic manipulation, this study evaluated the immature tassel response of 5 South Brazilian maize genotypes in 4 different culture media in relation to the capacity of embryogenic callus production and growth as well as the regeneration of plants from these calli.

Material and methods

The experiment was developed in the Plant Biotechnology Laboratory of the University of Passo Fundo. Maize genotypes were grown in the greenhouse at Embrapa Trigo in Passo Fundo, Rio Grande do Sul State.

Callus induction from maize immature tassels

Genotypes of simple maize hybrids Hs1 (PF96317/PF963244), Hs2 (PF963027/PF963244), Hs3 (PF972334/PF963004), Hs4 (PF972291/ PF963106) and Hs5 (PF972218/PF963173) belong to the Embrapa Trigo maize improvement program and were seeded in vases in greenhouses. To acquire immature tassels, 6-7-week-old plants (Figure 1a) were collected according to Songstad et al. (1992). Immature tassels measuring 2-3 cm (Figure 1b) were segmented (2-3 mm) and inoculated in petri dishes containing different callus-induction media.

Four types of culture media were used, including N6 (CHU et al., 1975) and MS (MURASHIGE; SKOOG, 1962) supplemented with different components: M1= N6 basic medium, 100 mg L^{-1} hydrolyzed casein, 2.88 g L^{-1} L-proline, Erickson vitamins, 10 µM $AgNO_3$, 100 mg L^{-1} myo-inositol, 20 g L^{-1} sucrose, 2 g L^{-1} phytagel and 1.0 mg L^{-1} 2.4-D (SONGSTAD et al., 1992); M2= equal to M1 medium without $AgNO_3$; M3= MS basic medium, 100 mg L^{-1} hydrolyzed casein, 2.88 g L^{-1} L-proline, Erickson vitamins, 10 µM $AgNO_3$, 100 mg L^{-1} myo-inositol, 20 g L^{-1} sucrose, 2 g L^{-1} phytagel and 1.0 mg L^{-1} 2.4-D; and M4= equal to M3 medium + 1 mg L^{-1} BAP (6-Benzylaminopurine). Erickson vitamins contain 0.5 mg L^{-1} thiamine HCl, 2 mg L^{-1} glycine, 0.5 mg L^{-1} pyridoxine HCL and 0.5 mg L^{-1} nicotinic acid. The pH of the media was adjusted to 5.8 with KOH (potassium hydroxide) before autoclaving (121°C for 20 min.). Next, $AgNO_3$ was sterilized via filtering and added to the culture medium.

A completely randomized experimental design was employed with 5 repetitions per treatment. The experimental unit was composed of one petri dish containing 10 segments of immature tassel. The cultures were maintained in the dark at 28±2°C for six weeks and subcultivated with the same fresh medium every 3 weeks.

After 42 days, embryogenic calluses were evaluated, weighed and transferred to maintenance medium for fresh mass evaluation. The maintenance medium had a similar composition to the induction medium, differing only in the absence of $AgNO_3$ and the increase in 2.4-D concentration to 2 mg L^{-1}. Cultures were maintained in the dark at 28±2°C and subcultivated every 21 days in maintenance medium to multiply embryogenic calli. The frequency and the fresh mass of embryogenic calli obtained at 42 days of cultivation were evaluated. The fresh mass was obtained from the average of calli obtained from 10 segments of immature tassels cultivated in a petri dish.

Calli were maintained *in vitro* for 8 subcultivations (21 weeks) to evaluate their longevity. Frequency data of embryogenic calli were transformed according to the arcsin formula ASEN [root((x+0.5)/100))*57.2958], submitted to variance analysis and the difference between the averages was compared using the Tukey test at 5%. Alternatively, data related to the fresh mass of embryogenic callus were compared using the average ± 1 standard deviation.

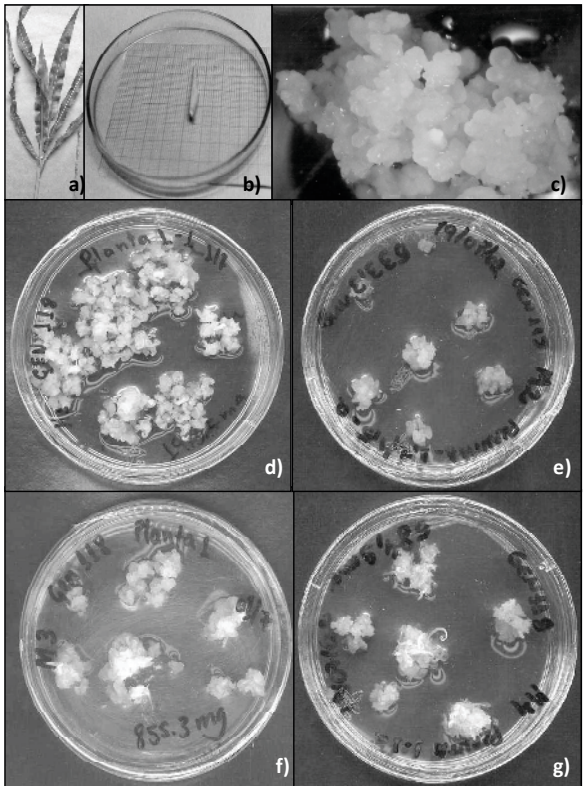


Figure 1. a) Maize plant at the immature tassel obtainment stage (6-7 weeks of cultivation); b) immature tassel used as an explant for callus induction; c) embryogenic callus of the Hs1 genotype; d) embryogenic calluses of the Hs1 genotype of maize during the maintenance stage originating from different callus induction media (M1 (d), M2 (e), M3 (f) and M4 (g)) after 7 subcultivations.

Maize plant regeneration from embryogenic calluses

Plant regeneration began after 21 weeks of induction of embryogenic calli, aiming at assessing the regenerative capacity of calluses maintained for a long time in cultivation. The regeneration system used was described by Sellmer et al. (1994). Embryogenic calluses were cultivated in regeneration medium (R1) composed of MS basic medium supplemented with modified White vitamins (0.2 g L^{-1} glycine; 0.5 mg L^{-1} nicotinic acid; 0.5 mg L^{-1} pyridoxine HCl; 0.5 mg L^{-1} thiamine HCl), 100 mg L^{-1} myo-inositol, 60 g L^{-1} sucrose, 2 g L^{-1} phytagel, pH 5.8. The cultures were grown for one week in the dark at 28±2ºC and then transferred to a growth chamber with a photoperiod of 16 hours light: 8 hours dark for 3 more weeks with ± 25 mmol m^{-2} s^{-1} lux illuminance. The calli were transferred to fresh medium of equal composition until the formation of shoots. The calluses/structures that presented shoots were transferred for rooting (R2) in medium similar to the regeneration medium but with sucrose reduced to 20 g L^{-1}.

Seedlings were removed from the medium and transferred to 500 ml pots containing Eucatex sterilized substrate buttonhole and vermiculite type substrate (1:1), and they were maintained for 4 days in a chamber with high humidity that was gradually decreased. After 7-10 days, seedlings were transferred to the greenhouse, initially protected from direct sunlight for 2-3 days and gradually exposed to the sun. The developed plants were transferred to 10 L vases with a Eucatex buttonhole and a vermiculite type substrate until they reached maturity to assess fertility and their morphological aspects.

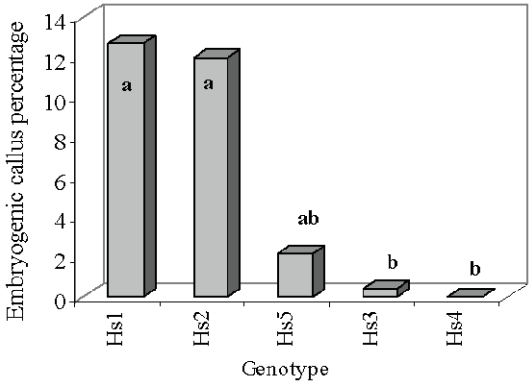
To evaluate the effectiveness of plant regeneration among the different genotypes, the number of shoots, complete seedlings (2-5 including root) and the number of acclimatized plants per gram of embryogenic callus transferred for regeneration medium were quantified. For example, one gram of fresh callus was homogenously distributed in the surface of a petri dish containing 25 ml of regeneration medium with 5 repetitions. Data were submitted to t-test at the 5% level of significance.

Results and discussion

Embryogenic calli induction

Of the 5 South Brazilian maize hybrids evaluated, 4 produced type I embryogenic calluses from immature tassel segments. The genotype influenced the frequency of the calli obtained ($p > 0.0007$); however, the culture media utilized did not influence the frequency of embryogenic calluses ($p > 0.3655$) (data not shown).

In total, 12.7 and 12% of calluses were produced by Hs1 and Hs2 genotypes, respectively, and these percentages were higher than the production rates of Hs3 and Hs4 genotypes (Figure 2). Figure 1c shows the characteristics of the embryogenic calluses that were classified as type I.



Averages followed by distinct letters differ by the Tukey test at 5% of significance.

Figure 2. Average percentage of embryogenic calluses obtained from immature tassels of different South Brazilian maize genotypes.

Genotype plays an important role in the formation of embryogenic calluses in different cultures, including maize. Genotypic differences regarding the formation of embryogenic maize cultures from other maize explants have been extensively reported in the literature (FERNANDES et al., 2008; FRAME et al., 2006; ISHIDA et al., 2007; LEE et al., 2007; TOMES; SMITH, 1985).

The expression pattern during somatic embryogenesis in different maize genotypes is controlled by a gene or a block of genes (WILLMAN et al., 1989). Hodges et al. (1986) suggest that nuclear genes exhibiting dominance are important for the formation of somatic embryos or plant regeneration in maize. Alternatively, Tomes and Smith (1985) reported that the genetic heritage control in maize involved in embryogenic callus formation is the additive type, with a significant maternal effect and heterosis. Armstrong et al. (1992) found RFLP markers associated with chromosomes 1, 2, and 9, and the most critical region for callus formation is in the long arm of maize chromosome 9. These authors proposed the existence of a primary gene (or genes) in this region that promotes embryogenic callus initiation and plant regeneration. Recently, this group of researchers (LOWE et al., 2006) developed a molecular marker-assisted selection methodology to assess embryogenic callus initiation in maize.

According to Krakowsky et al. (2006), the genetic basis for the production of maize regenerative calluses is not completely elucidated. These authors discovered the existence of 11 QTLs in 8 chromosomes, 8 QTLs showed a main effect and 3 presented an epistatic interaction.

The obtainment of genetically modified plants greatly depends on the capacity of the *in vitro* response of the genotype used. Nevertheless, some maize genotypes, especially elite material, present a poor tissue culture response, which limits the number of genotypes that were effectively transformed (FRAME et al., 2006; ISHIDA et al., 2007; LEE et al., 2007). The Hi-II hybrid, due to its high *in vitro* response, has been used for genetic transformation; however, it has poor agronomic characteristics. Thus, the application of this technology in agriculturally relevant genotypes, such as locally developed hybrids by genetic breeding programs, is highly desirable (FRAME et al., 2006; GORJI et al., 2011; HUANG; WEI, 2005; ISHIDA et al., 2007; LEE et al., 2007), which justifies the selection of *in vitro* responsive genotypes.

The purpose of this study is to evaluate the *in vitro* response of Brazilian genotypes and alternative explants that can be used in tissue culture and maize genetic transformation.

In maize culture, the immature zygotic embryo is the most widely used explant for the establishment of embryogenic callus cultures and genetic transformation (FRAME et al., 2002, 2006, 2011; ISHIDA et al., 1996, 2007; LEE et al., 2007; VEGA et al., 2008) because the immature embryo presents cells that are competent for somatic embryogenesis, unlike other tissues that are more differentiated. However, the production of immature embryos is a demanding process that requires time and painstaking activities for the growth of plants and pollination. Thus, the use of more readily available alternative explants, such as immature tassels, can accelerate the obtainment of *in vitro* plants.

Maize immature tassels were used by Songstad et al. (1992) to acquire embryogenic cultures in model genotypes such as A188 and B73 lines and its Hi-II hybrid. Embryogenic calli obtained from immature tassels have been used to introduce genes into this hybrid via bio-ballistics (HE et al., 2003; GRANDO et al., 2005). Rhodes et al. (1986) evaluated 13 North American maize genotypes in relation to their ability to start embryogenic calluses from immature tassels, which determines the genotypic effect for this characteristic. They observed that not all genotypes expressed the capacity of regenerating *in vitro* plants and that, within the genotypes, *in vitro* responses of immature embryos and tassels were qualitatively and quantitatively similar.

Fresh mass of embryogenic calli

The fresh mass of the embryogenic calluses was evaluated at 42 days of culture. On average, Hs1 genotype calli produced a greater amount of fresh mass (670 mg) when compared to other genotypes (10 and 70 mg) (Table 1).

Table 1. Fresh mass of embryogenic calli acquired from immature tassels of 4 maize hybrids cultivated for 42 days in 4 culture media.

Genotype	Media	Fresh mass (mg)*	
Hs1	M1	670	S
	M2	500	
	M3	860	S
	M4	640	S
Average		670	
Hs2	M1	10	
	M2	20	
	M3	190	
	M4	60	
Average		70	
Hs3	M1	40	
	M2	-	
	M3	-	
	M4	-	
Average		10	
Hs5	M1	-	
	M2	20	
	M3	160	
	M4	20	
Average		70	
	General Average	270	
	Standard deviation	310	

Average + standard deviation = superior (S); Average – standard deviation = inferior (I).

Although the culture media did not influence the percentage of embryogenic calluses, the media did influence the fresh mass of induced calli. Regarding the Hs1 genotype, the calli cultivated in M1, M3, and M4 (containing silver nitrate) media were considered superior to other treatments.

The media M3 (MS basic medium) and M4 (MS+BAP basic medium) promoted the growth of embryogenic calli of the Hs1, Hs2 and Hs5 genotypes; however, the calli maintained in these media through 8 subcultivations (21 weeks) lost their embryogenicity and presented differentiated structures in the form of leaves and shoots (Figure 1g and f). These observations are in agreement with Vasil (1987), who reported that embryogenic calluses cultivated in MS medium may become organogenic or germinate early.

However, the calli induced in M1 and M2 media (N6 basic medium) maintained their embryogenic features for approximately 5 months of culturing (Figure 1d and e). Normally, the embryogenic calli of cereal species are characterized by slow growth because they are unable to be cultivated during a long period of time (NABORS et al., 1983). This experiment shows that the use of N6 basic medium allows embryogenic calli to be kept for a longer period compared to MS medium. Similarly, Gorji et al. (2011) observed that maize embryogenic calli induced in N6 medium were of better quality than calli obtained in MS medium.

MS medium has been more widely used for the induction of type I maize embryogenic calli (FRAME et al., 2006), and N6 medium has been used for the induction of type II embryogenic calli, primarily the A188 genotype and its hybrids, including Hi-II (FRAME et al., 2002, 2011). These findings support the need to evaluate the behavior of the interaction between genotype and the culture medium. In this experiment, even using alternative genotypes, N6 basic medium fostered the formation of type I embryogenic calli of best quality.

The various responses observed during the production of somatic embryos and maize calluses in MS and N6 media can be attributed to its different nitrogenous compounds (ARMSTRONG; GREEN, 1985). MS medium contains 60 mM nitrogen (20.6 mM NH_4^+ and 29.4 mM NO_3^-) compared with N6 medium, which contains 35 mM nitrogen (7 mM NH_4^+ and 28 mM NO_3^-). The ammonia: nitrate ratio is approximately 1:2 in MS medium; however, it is 1:4 in N6 medium. The form of nitrogen may have a fundamental role in the expression of genes that control the embryogenic process because this difference is the greatest between N6 and MS media (HODGES et al., 1986).

In this study, N6 basic medium fostered a greater proliferation of embryogenic calluses throughout successive subcultivations when silver nitrate was added. Figure 1d and e shows the greater proliferation of embryogenic calli cultivated in M1 medium (N6+$AgNO_3$) compared to calli cultivated in M2 medium (N6 without $AgNO_3$) after 8 subcultivations.

Silver nitrate has been used to increase the production of type II calli and the regeneration of maize plants. The addition of 10 µM $AgNO_3$ in culture medium increased the production of embryogenic calli in 59% of Hi-II hybrid maize immature tassels (SONGSTAD et al., 1992). The effect of $AgNO_3$ is related to its action as an ethylene antagonist, which prevents the formation of endogenous ethylene that is generated by mechanical damage caused to the explants by the process of in vitro cultivation and affects the embryogenic response.

The importance of evaluating the effects of genotypes and culture media throughout subcultivations is justified by the need of the calluses passing through a long period in selection medium during the genetic transformation process. Thus, the embryogenicity of these calluses should be maintained during this period to ensure the regeneration of genetically modified plants.

Maize Plant regeneration from embryogenic calluses

Only Hs1 calli induced in M1 and M2 media that remained embryogenic throughout subcultivations were evaluated for regeneration potential. The culture medium used in callus induction did not influence plant regeneration. This genotype produced approximately 11.7 shoots, 4.9 seedlings, and 3.3 acclimatized plants per gram of callus transferred to the regeneration medium (Table 2).

Table 2. Average shoot production, 2-5 cm rooted seedlings, and acclimatized plants per gram of embryogenic callus of the Hs1 genotype of maize cultivated in regeneration medium.

CIM	NS	NSd	NAP	NRP
M1	8.9 ns*	4.0 ns	3.2 ns	29
M2	14.5	5.8	3.3	13
Average/total	11.7	4.9	3.3	42

CIM = callus induction media; NS = number of shoots per gram of embryogenic callus; NSd = number of seedlings per gram of embryogenic callus; NAP = number of acclimatized plants per gram of embryogenic callus; NRP = number total of regenerated plants; *ns= not significant according to t-test in 5% level of significance.

Approximately, 42% of the shoots produced roots that formed seedlings, and 69% of these were acclimatized. The plants reached maturity and became fertile. Figure 3 displays the stages of the process of plant regeneration and acclimatization of the Hs1 maize hybrid.

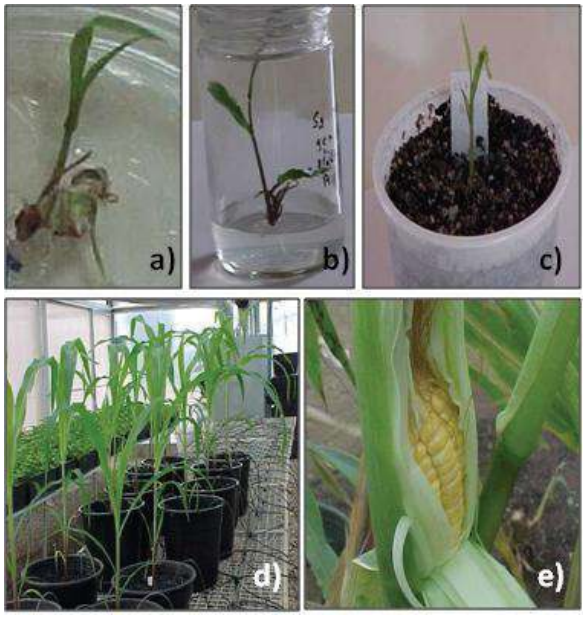


Figure 3. Stages of *in vitro* plant regeneration of Hs1 maize. a) somatic embryo germination in R1 medium; b) maize seedling development in R2 medium; c) seedling acclimatization in vase with substrate in high humidity environment; d) plant development in greenhouse; e) ear of corn showing the fertility of *in vitro* regenerated plants.

In this study, *in vitro* plants of a South Brazilian maize genotype were regenerated from embryogenic calli induced from immature tassel explants. Plant regeneration from indirect somatic embryogenesis is a prerequisite for the production of genetically modified plants, mainly in cereals (FRAME et al., 2002, 2006; ISHIDA et al., 2007). The identification of productive genotypes that are adapted to environmental conditions and that also present the capacity of responding *in vitro* allows genetic transformation to be performed with genotypes alternative to the Hi-II model genotype.

The Hi-II genotype has been internationally used to produce maize transgenic plants; however, this genotype presents low agricultural value, which increases the time needed as well as the costs for transgene transference to locally adapted lines by backcrossing (LUPOTTO et al., 2004). Furthermore, backcrossing is hindered by the incompatibility of heterotic groups and low combination capacity (O'KENNEDY et al., 2001).

The use of the immature tassels accelerates the obtainment of *in vitro* plants due to its higher speed of development in comparison to the immature embryo. The tassel becomes available 6-7 weeks after seeding, but the immature embryo can only be collected 14 weeks after seeding. In addition, artificial pollination remains necessary.

Conclusion

The immature tassel has proven to be an effective alternative explant in the induction of embryogenic calli and the regeneration of *in vitro* maize plants.

A South Brazilian maize genotype (HS1) and a culture medium (N6 basal medium + $AgNO_3$) that permit induction, proliferation and longevity of embryogenic calli were identified, enabling the regeneration of complete and fertile maize plants. These parameters can be used in experiments of genetic manipulation aiming at the obtainment of genetically modified plants.

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Received on December 16, 2011.
Accepted on March 16, 2012.