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Faculty of Biology and Geology
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Sumary of Doctoral Thesis

STUDIES REGARDING SOME FACTORS INVOLVED IN SUNFLOWER PROTOPLAST AND TISSUE EXPLANT ORGANOGENSIS AND SOMATIC EMBRYOGENESIS

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Keywords: sunflower, *Helianthus annuus*, *in vitro* regeneration, organogenesis, somatic embryogenesis, protoplast, haemoglobin, *gfp, Agrobacterium tumefaciens*

1. INTRODUCTION

The sunflower is one of the most important oil-producing crops being on the second place in Europe after rapeseed and on the fourth place in world after palm tree, soybean and rapeseed (Honda et al., 2005; Fernandez-Martinez et al., 2007). In our country, until the 1990s sunflower was cultivated on more then 900 thousand ha (Vrânceanu, 2000). Because its economical value represents an important target for biotechnological research - interspecific fusion of the protoplasts and gene transfer techniques represents new ways for transferring valuable traits in sunflower genome. The first step for implementing such methods is the improving of the *in vitro* regeneration capacity of the sunflower (Pugliesi et al., 1993; Fiore et al., 1997). Sunflower is recognized for its poor *in vitro* regeneration among agronomical important plants (Freyssinet şi Freyssinet, 1988). Regeneration frequency depends on genotype and most genotypes are reported to be recalcitrant (Deglene et al., 1997). Therefore new researches are necessary for establishing efficient plant regeneration methods starting from different tissues and protoplasts (Alibert et al., 1994).

Several progresses have been recently made regarding the *in vitro* sunflower regeneration but even so some issues still exist. These problems can now be resumed as: low reproducibility of the regeneration protocols which are genotype-dependant, inefficient rooting of the *in vitro* regenerated plants, and premature *in vitro* flowering (Power, 1987; Baker et al., 1999; Fauguel et al., 2008).

Until now the most reliable explants source - with high regeneration potential for many genotypes - is the immature zygotic embryo. The great disadvantage of this biologic material is the difficulty of obtaining it. Therefore the necessity of finding a regeneration method based on a starting tissue which is easy to be obtained all year round can be considered as particularly important. Having all these aspects into consideration our goal was to establish a successful *in vitro* regeneration method applied for several genotypes with high agricultural value. For these genotypes the earlier established methods (Paterson and Everett, 1985) did not produce satisfactory results (Aurori et al., 2000).

One of the main goals of this thesis was to find the most efficient ways to improve *in vitro* sunflower regeneration by starting from different types of explants. These studies included the role of explant age upon *in vitro* sunflower regeneration efficiency as this factor was always considered critical for this species. Also, the influence of the explant type on the *in vitro* regeneration was taken into consideration, using as starting material explants from mature ungerminated zygotic embryos. Previously, these tissue fragments were not frequently used for *in vitro* culture of sunflower. The most efficient regeneration methods which were found during these studies were subsequently applied for *in vitro* culture of several sunflower hybrids with

important agronomic traits. Also, a method to obtain fertile plants by *in vitro* tissue culture was established for Turbo hybrid.

The embryonic meristematic dome, an explant which wasn't used for *in vitro* sunflower cultivation until now, was subjected to study regarding the role of different auxins (IAA, IBA, NAA, 2,4-D, dicamba şi picloram) upon regeneration via organogenesis or somatic embryogenesis. Also, the role of sucrose concentration upon morphogenetic processes was studied in relation to the type of auxin used in culture media. A special attention was given to the aspects regarding the morphology of the regenerated somatic embryos.

The oxygenation of the protoplasts in liquid culture medium may be a problem due to their fragility and therefore to the impossibility of their agitation. From these reasons their capacity to divide and to follow the development in culture may be affected. Considering these reasons the finding of some ways to improve the oxygenation of the immersed protoplasts was necessary. The perfluorocarbons and haemoglobin are some compounds with the ability to enhance oxygen concentration in liquid medium. This was previously demonstrated for cell and protoplasts cultures of other species than sunflower (Anthony et al., 1997b). Therefore, another goal of our study was to investigate the effect of haemoglobin in the liquid culture medium of sunflower protoplasts at protoplast plating efficiency level and at cell colony formation as well.

Preliminary investigations regarding the best ways to improve genetic transformation of sunflower by using *Agrobacterium tumefaciens* carrying the reporter gene *gfp* were performed as well. This gene was not used until now for genetic transformation of mature tissues of sunflower. Previously, only immature zygotic embryos were transformed with *gfp* gene (Müler et al., 2001). Several wounding methods were approached in order to improve genetic transformation efficiency of sunflower by using mature embryonic axis as starting material.

2. MATERIAL AND METHODS

. The first goal of this study was to investigate the role of the age and the type of the explants upon *in vitro* sunflower regeneration. The studies were carried out on the Florina hybrid and in the first step the role of the age of the explants were taken into consideration by comparing the *in vitro* response of the embryonic axis and the fragments of the cotyledon resulted from ungerminated zygotic embryo or from germinated embryo, having different days after germination (1-6 days). Also a comparative analysis of the morphogenetic response of different explants originated from mature sunflower embryos was made.

Several sunflower hybrids (Turbo, Florom 328, Select, HS2411, Alcazar, Rapid, Coril, Santiago, Felix, Splendor, Top 75, Florina şi Romina) and 47320bcd French line were tested for *in vitro* sunflower regeneration using mature embryonic axis as explants. In the case of Turbo hybrid, after *in vitro* regeneration of the plants they were rooted on different culture medium and *ex vitro* acclimatized afterwards.

Using embryonic meristematic dome the role of auxin IAA, IBA, NAA, 2.4-D, dicamba, picloram upon *in vitro* morphogenetic process was analyzed. Several sucrose concentrations (3%, 6%, 9%. 12%) were added to the culture medium in order to improve somatic embryo development.

The morphogenesis parameters taken into consideration were the regeneration ability representing the number of explant giving shoots (or embryos) per 100 explants plated and the regeneration efficiency representing the average number of shoots per regenerating explant.

The effect of haemoglobin on protoplast plating efficiency and subsequently on the number of cell colony formation was investigated by adding this compound into the liquid culture medium of the protoplasts.

Also, as was previously mentioned, we tested different ways to improve genetic transformation of mature embryonic axis by using *Agrobacterium tumefaciens* as transformation agent, carrying the genetic construction pHB2892 that contains *nptII* marker gene for kanamycin resistance and *gfp* reporter gene for green fluorescent protein synthesis (Molinier et al., 2000; Rakosy-Tican et al., 2000).

3. RESULTS AND DISCUSSIONS

3.1. *In vitro* organogenesis of sunflower: the role of the explants age

Organogenesis in sunflower is considered to be greatly influenced by the developmental stage of the explants (Deglene et al., 1997; Espinasse and Lay, 1989).

The only explant allowing reproducible results with a wide range of genotypes is the immature zygotic embryo although the technique requires considerable time and effort (Hewezi et al., 2003; Power, 1987; Finer, 1987; Sujatha and Prabakaran, 2001). By different methods it was demonstrated that the explants resulted from plantlets loose their regeneration ability compared with those resulted from embryos (Power, 1987; Finer, 1987). The general conclusion is that the morphogenetic capacity is declined rapidly with increasing of the age of the explants (Knittel et al., 1991).

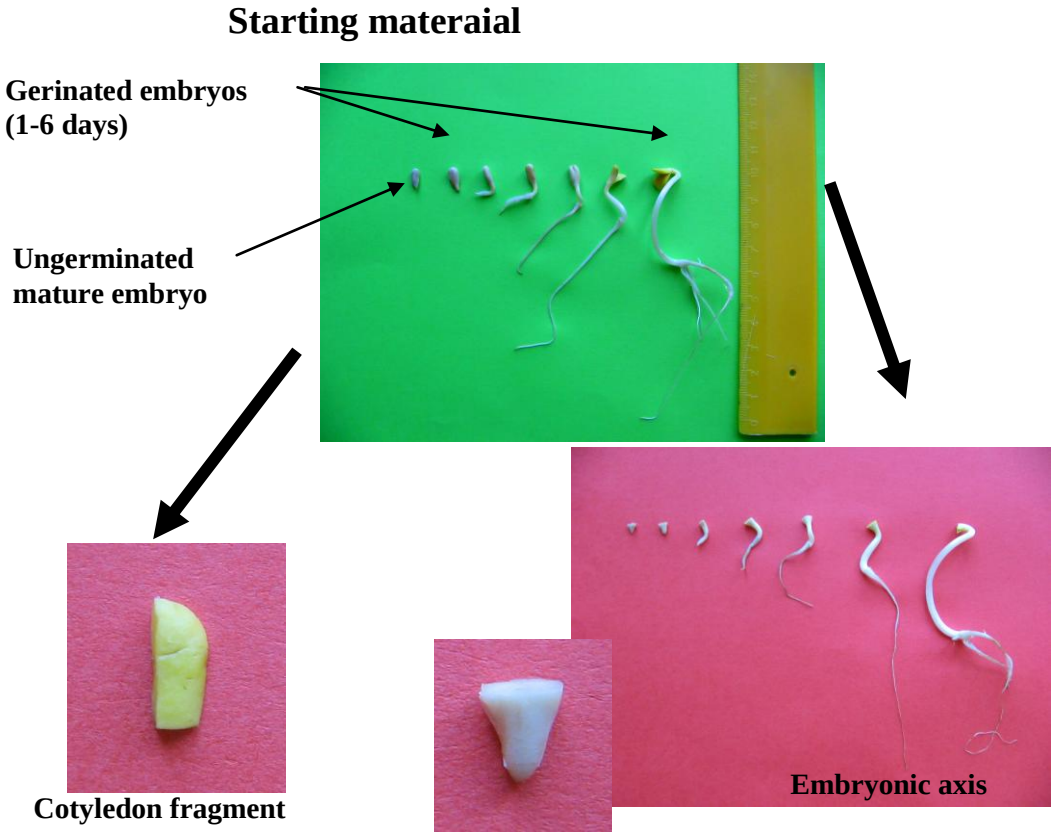

Fig. 1 The starting material represented by the embryonic axis and the cotyledon fragments resulted from ungerminated mature embryo or germinated embryos (1-6 days)

Up to date, the data regarding the regeneration of explants resulted from mature ungerminated embryos are still poor. Having these aspects into consideration in this report we compared the regeneration ability of the embryonic axis and the cotyledon fragments resulted from mature ungerminated embryos or from germinated embryos having different ages (1-6 days) (Fig. 1). The experiments were carried out on Florina hybrid.

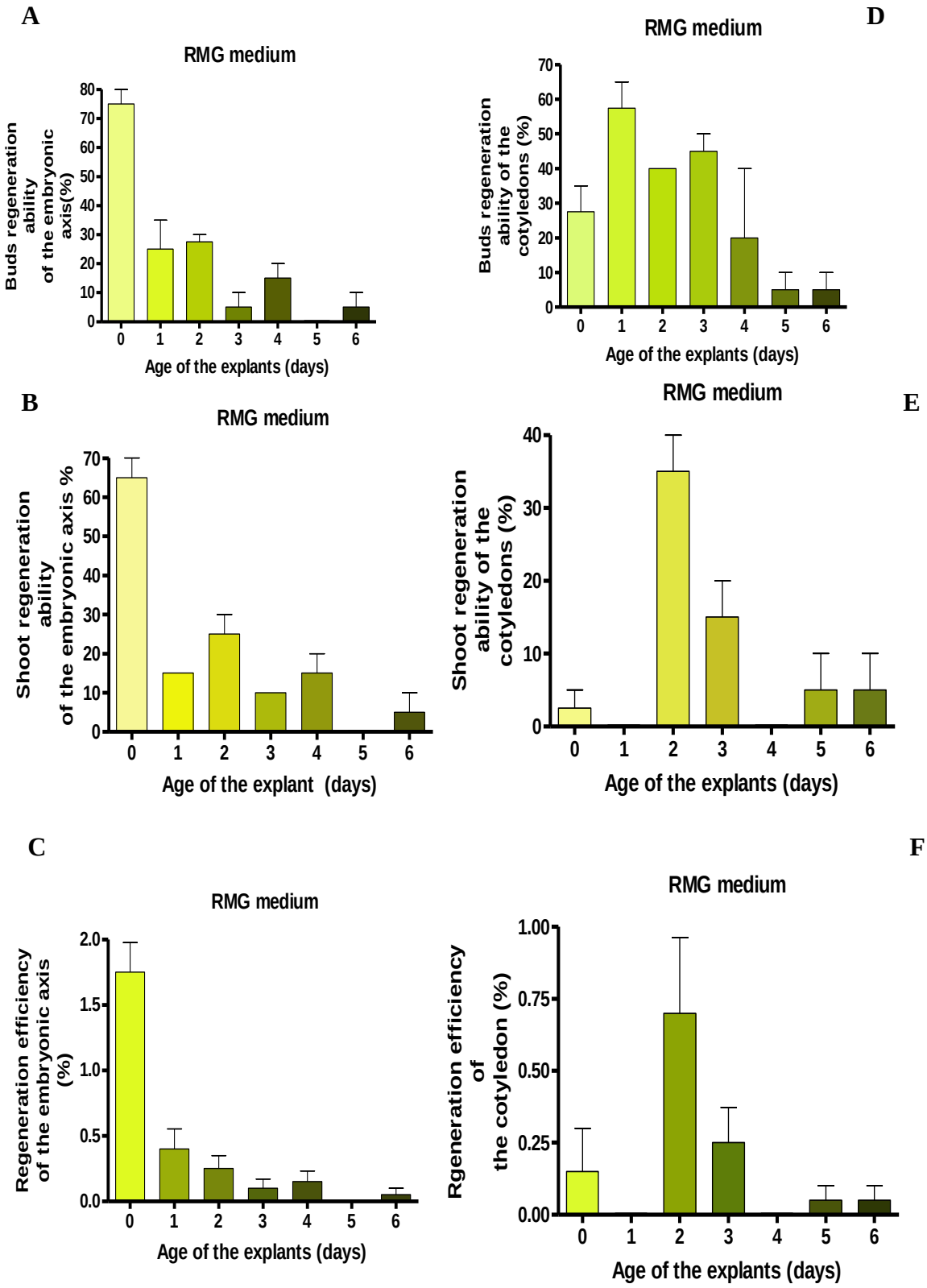

Fig. 2 The role of the age of the explants on plant regeneration efficiency in sunflower embryonic axis and cotyledons, cultivated on RMG medium

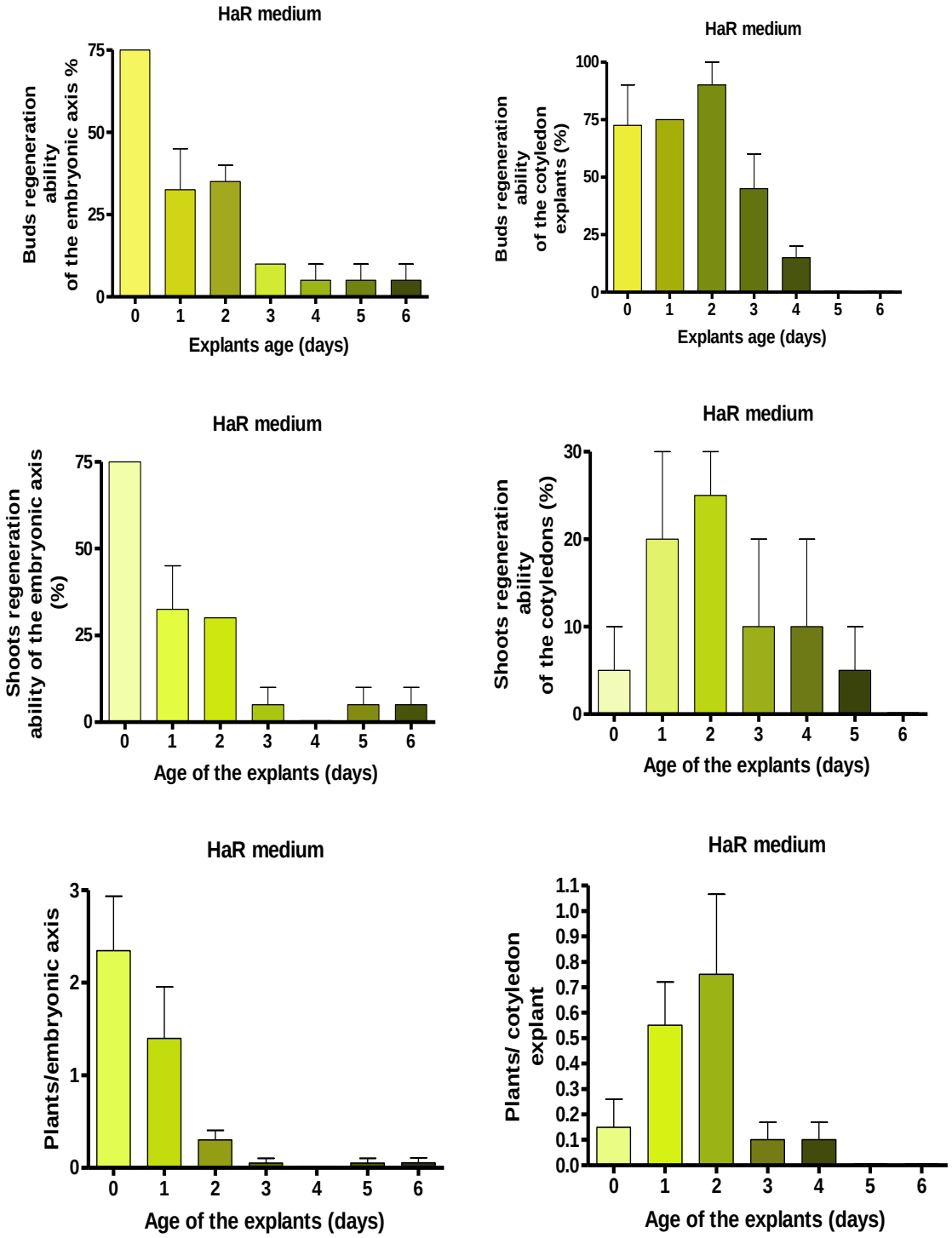

Fig. 3 The role of the age of the explants on plant regeneration efficiency in sunflower embryonic axis and cotyledons, cultivated on HaR medium

The embryonic axis in the form in which it was used contained the two meristems – apical and radicular - the primary leaves, and about 1 mm of the cotyledonary base. In order to germinate the seeds were cultivated on 50 % concentration MS salts medium (Murashige and Skoog, 1962). During the germination process the hypocotyls of the embryonic axis are

elongating. Two culture media were used – RMG (Rakosy-Tican, 1998) and HaR (Paterson and Everett,1985). A two step procedure for *in vitro* regeneration of the explants was developed consisting in shoot inducing NAA and BAP containing medium and shoot development medium containing BAP and GA3. On the inducing step the cultures were maintained for 4-10 days in the dark and after that they were transferred to the light.

The induced buds were visible to the naked eye as early as 4 days after the explants were cultivated on regeneration medium (Fig. 4). The age of both kinds of explants was critical for their regeneration ability but each type of explant had different requirements. The embryonic axis explants had the best response when they resulted from ungerminated embryos, contrary the cotyledon explants which reacted better when they were originating from 2 days germinated embryos (Fig. 2 and Fig 3).

Organogenesis occurred directly for both type of explants. After two weeks since the tissue culture was initiated the potential of shoot regeneration was evaluated considering the percentage of explant giving shoots and the mean number of shoots regenerated per explant. The general observation was that although a great number of explants produced buds only a small percentage of them are capable to regenerate shoots.

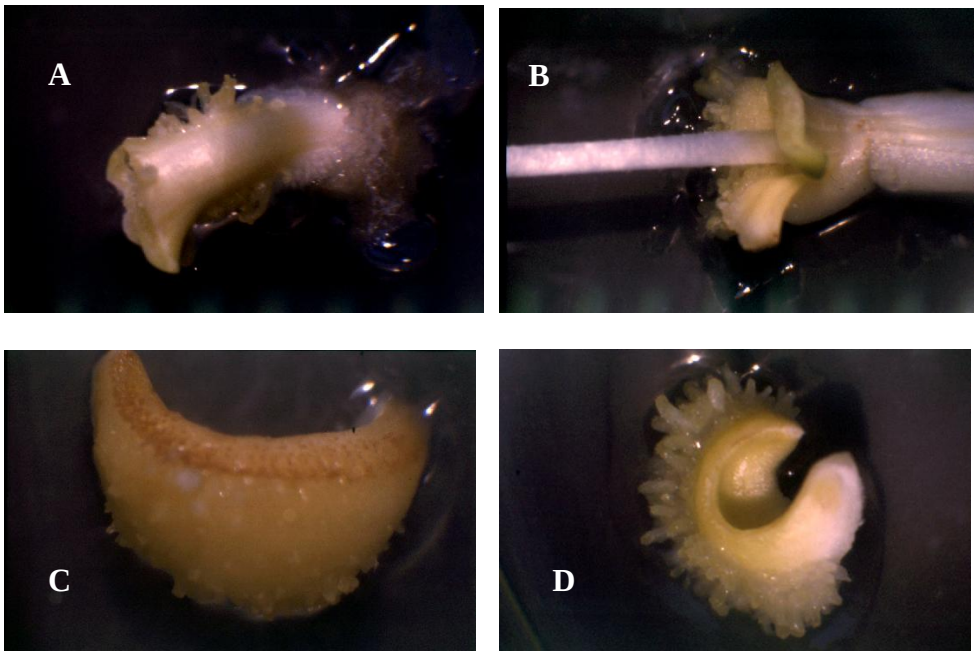

Fig. 4 The shoots regenerated on the sunflower embryonic axis (A, B) or on the cotyledon fragments (C, D) on RMG culture medium (A, C) or HaR culture medium (B, D), in Florina hybrid

The only explant type that keeps unchanged its potential for regenerating shoots compared to the potential of regenerating buds is the mature embryonic axis.

The different types of culture media which were tested did not have a significant effect upon the regeneration efficiency.

In conclusion, the best *in vitro* response was obtained from embryonic axis resulted from ungerminated mature embryos.

3.2. The role of the explant type on *in vitro* organogenesis of sunflower

In order to establish the best response of ungerminated mature embryos for *in vitro* morphogenesis, 6 types of explants were compared (Fig. 5). Three of them resulted from embryonic axis (E1, E2, E3) and another three resulted by dividing cotyledons in two ways, lengthwise and transversal, respectively (E4, E5, E6). *In vitro* cultivation was performed as was described in the previous study.

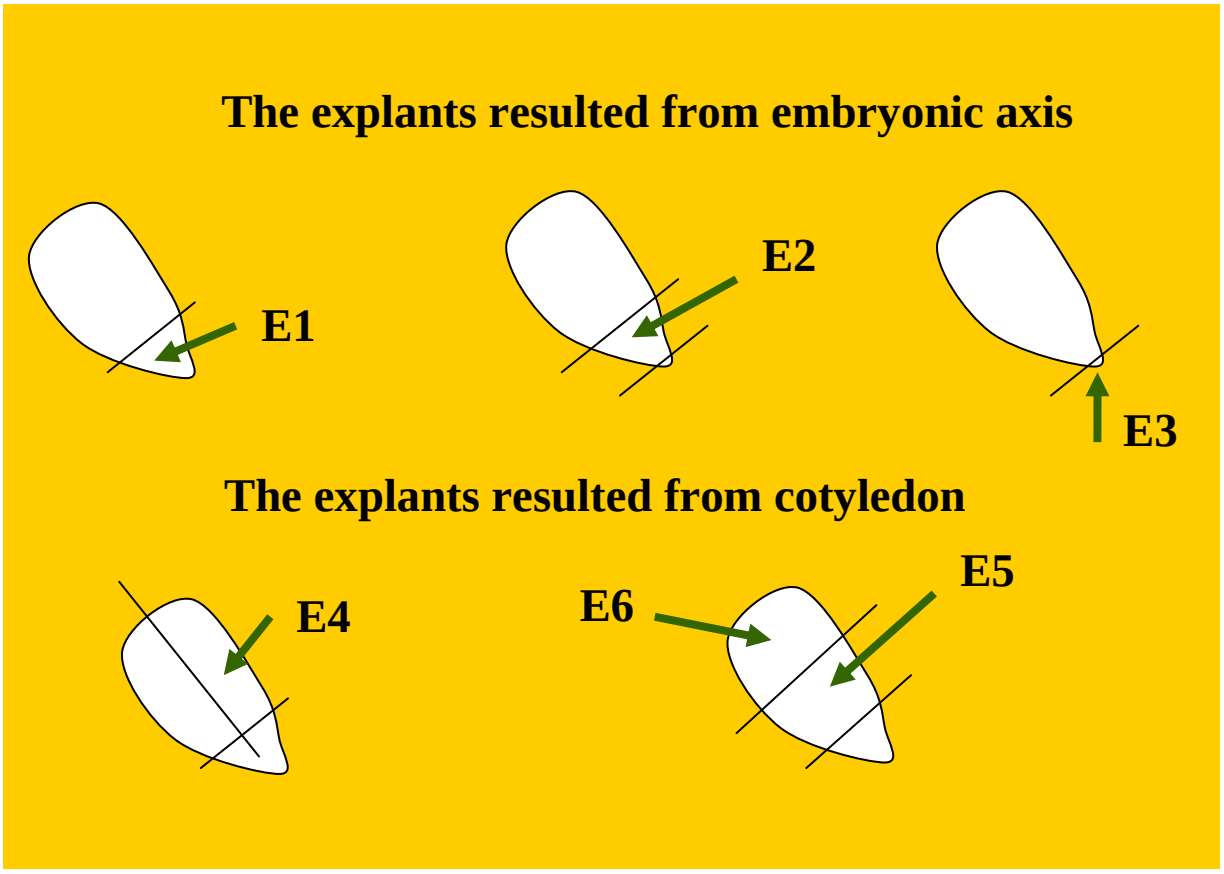

Fig. 5 The embryonic axis and the cotyledons explants resulted from ungerminated mature sunflower embryos

Excepting the E3 explant who regenerated only callus, and the E4 who had a poor *in vitro* development, all the others had a good morphogenetic response, up to 60% of the explants regenerating buds. As we previously observed, only the E1, E2 explants types (on RMG and HaR medium) and E5 (on HaR medium) are capable to sustain the development of the buds up to shoots stage (Fig. 6). The efficiency of plant regeneration followed the same pattern.

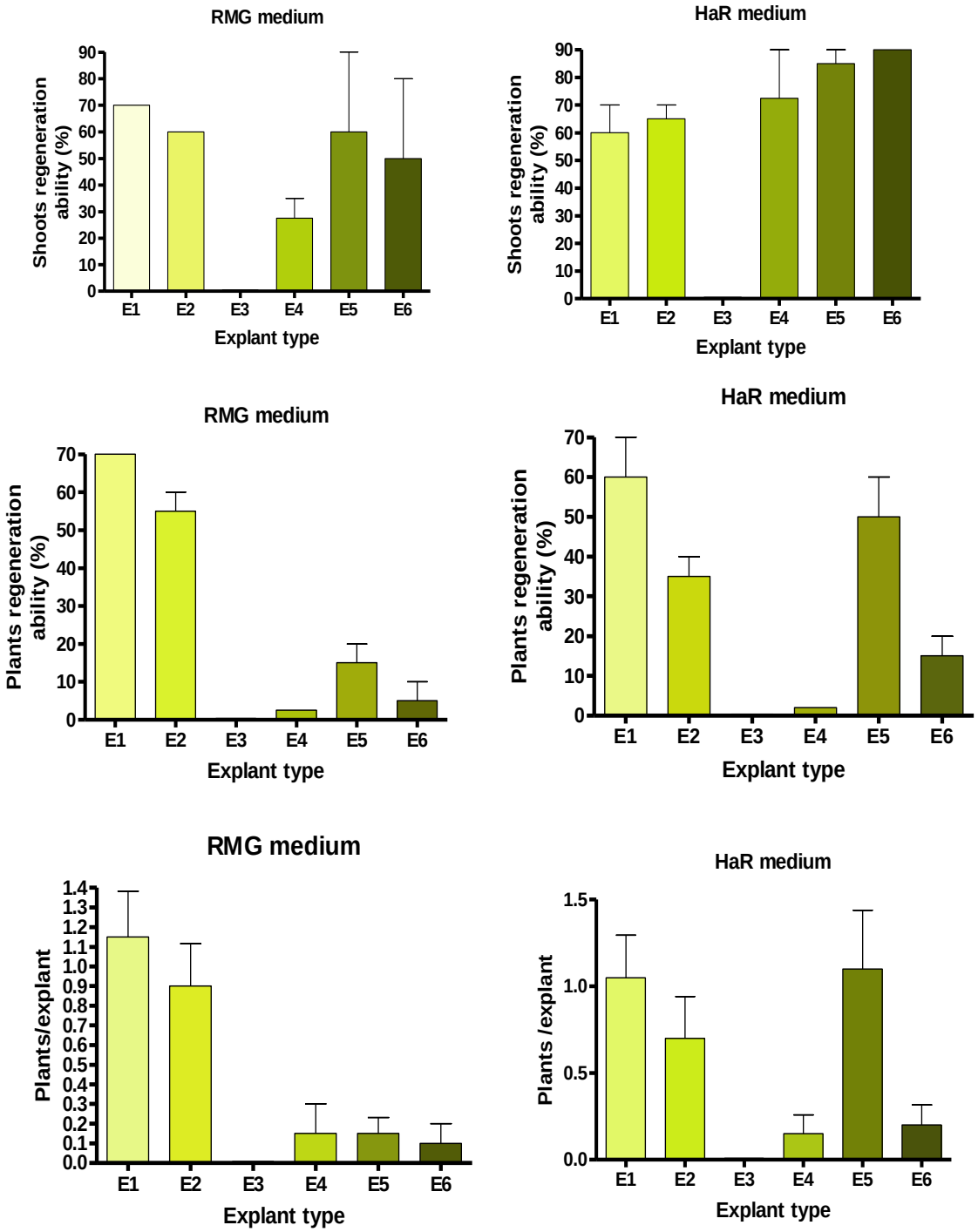

Fig. 6 The role of the explant type on plant regeneration efficiency on RMG or HaR culture media in sunflower, Florina hybrid

The cotyledons are considered explants with a high regeneration potential in sunflower (Knittel et al., 1991; Baker et al., 1999). Because of the large number of regenerated buds on the cotyledon explant a competition may be created between them and their development could suffer (Christianson and Warnick, 1984). This fact was confirmed in our study also. Moreover we demonstrated that the intact embryonic axis have even a better response.

We can conclude that the most efficient explants for plant regeneration are the intact embryonic axis which possess both meristems – radicular and apical (E1) and the proximal half of the cotyledon as well(E5).

3.3. Plant regeneration from mature embryonic axis of different sunflower genotypes. A reliable protocol of regenerating fertile plants for hybrid Turbo

It has been demonstrated that plant regeneration response is under genetic control in sunflower (Deglene et al., 1997) and the most genotypes are reported to be recalcitrant (Power, 1987; Witrzens et al., 1988; Deglene et al., 1997).

Fourteen genotypes were used in this study: thirteen hybrids (Turbo, Florom 328, Select, HS2411, Alcazar, Rapid, Coril, Santiago, Felix, Splendor, Top 75, Florina and Romina) and one inbred line (47320bcd). The hybrids were selected on the basis of their important agronomic traits (Vrânceanu, 2000). The regeneration method based on using mature embryonic axis was previously described. Mature embryonic axis has a good regeneration potential. The expression of this potential is influenced by a number of diverse factors. In order to improve the regeneration efficiency the explants were cultivated at higher temperature (28-30 ^{o}C). Also the main step in the regeneration process was the cutting of the developing shoots from the apical meristem after the adventitious buds become visible. The phenomenon, generally known as apical dominance was also observed in sunflower tissue culture by Hewezi et al. (2003). The regeneration was further stimulated by cutting the *de novo*-formed shoots.

All the genotypes, excepting the line 4732bcd, have shown very high regeneration ability, 85-100% of the explants being able to regenerate shoots (Fig. 7). Regarding the number of plants/regenerating explant the genotype can be divided in three categories: 1) with a very high regeneration potential giving a mean number of 30-40 plants/explant (Select and Turbo); 2) the second category with a good regeneration potential (Florom 328 and HS4211); 3) the third category regenerating a mean number of 10-20 plants/explant (Fig. 8). The shoots are regenerated in different points on the embryonic axis i.e. peri-meristematic, the hypocotyls, the base of the cotyledons, the epicotyls and the primary leaf (Table 1).

Another goal of this study was the development of a method for obtaining fertile plants for hybrid Turbo. Three culture media were tested: RMG, HaR and MSc at different pH values (5.5, 5.8, 7). The rooting efficiency of regenerated shoots was tested on different culture media: MS, MS with 0.1 mg/L IBA or MS with 0.3% activated charcoal.

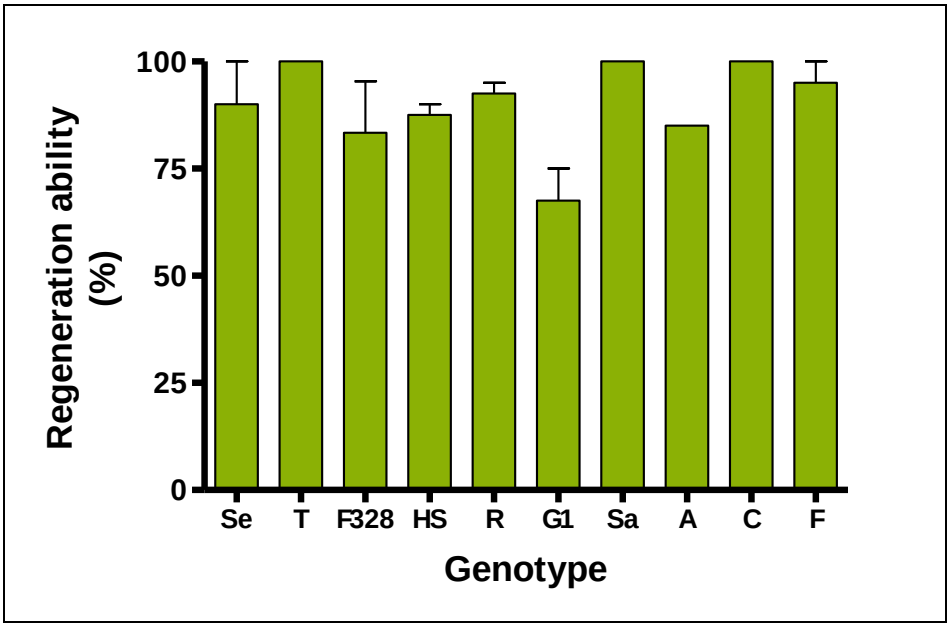

Fig. 7 The plant regeneration ability of different sunflower genotypes: (T - Turbo, F328 - Florom 328, Se-Select, HS - HS2411, A - Alcazar, R - Rapid, C- Coril, S - Santiago, F- Felix, G1 - 47320bcd

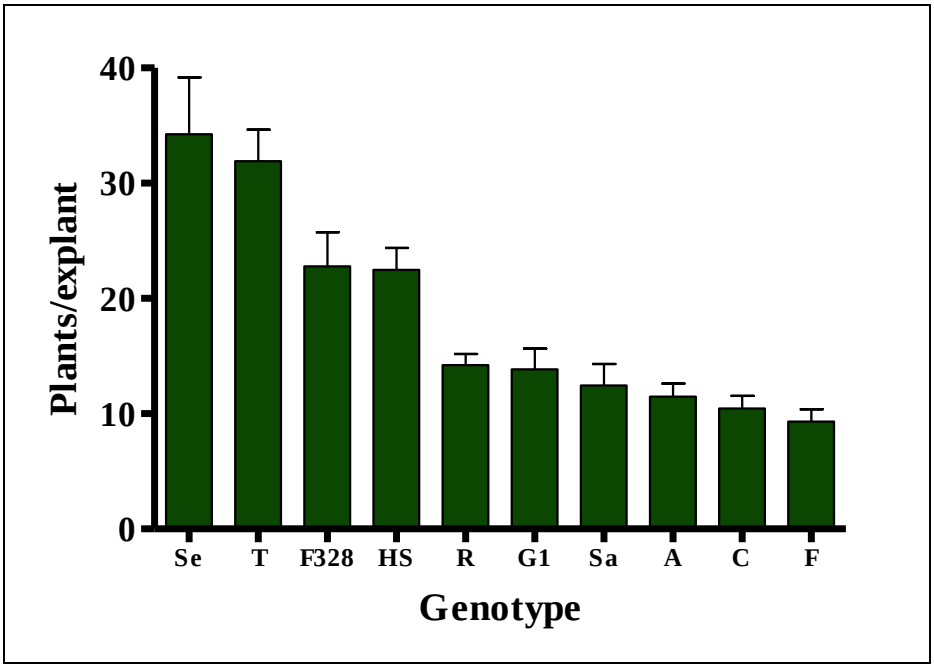

Fig. 8 The plant regeneration efficiency of different sunflower genotypes: (T - Turbo, F328 - Florom 328, Se-Select, HS - HS2411, A - Alcazar, R - Rapid, C- Coril, S - Santiago, F- Felix, G1 - 47320bcd

Table 1. The plant regeneration efficiency of the hybrids: Splendor, Top75, Florina, Romina; the plant regeneration areas

Hybrid	Total regenerated plants	Areas of plant regeneration				
		Peri-meristematic	Hypocotyls	Cotyledon	Epicotyls	Primary leaf
Splendor	16	9	-	2	-	5
Top75	69	4	7	43	14	1
Florina	25	19	6	-	-	-
Romina	8	3	-	-	3	2

For Turbo hybrid the regeneration medium wasn't the critical factor for regeneration efficiency but had an influence on further development of plants (Fig. 9). Plants resulted from HaR medium, pH 5.5, were the most vigorous and had the best rooting potential. Also, the most efficient rooting medium was MS with 0.1 mg/l IBA (Fig. 10).

The plants with well developed roots were successfully acclimated *ex vitro* and did set flowers that formed mature seeds (Fig. 12).

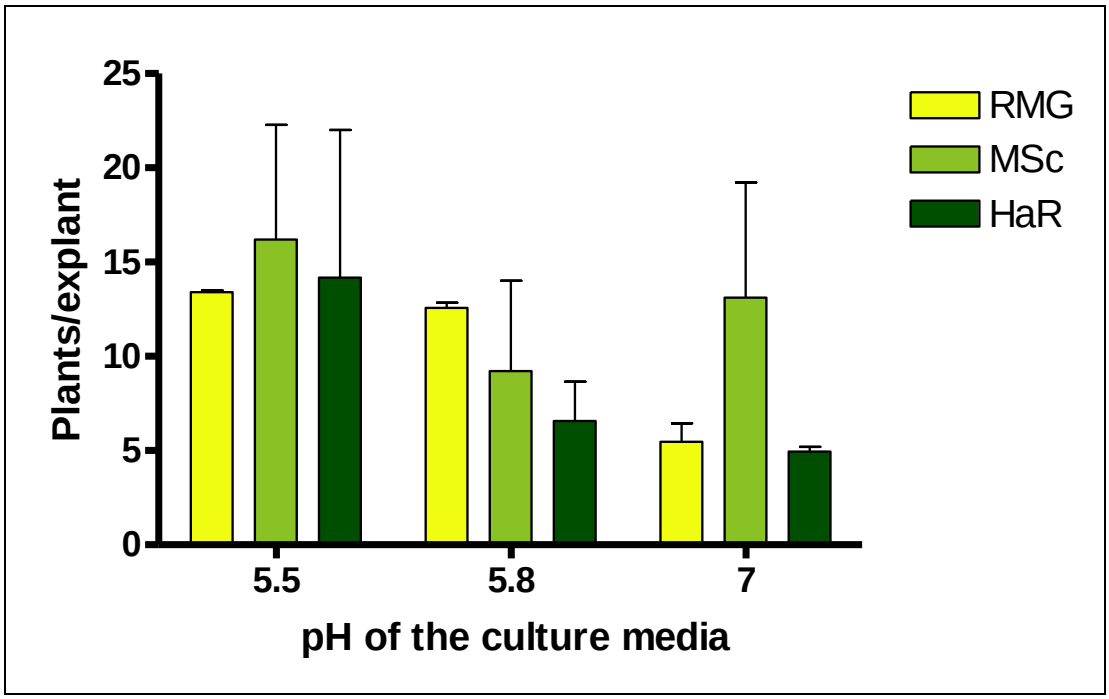

Fig. 9 Plant regeneration efficiency of sunflower embryonic axis, Turbo hybrid, on three culture media (RMG, MSc, HaR) and different pH values of the culture media (5.5, 5.8, 7)

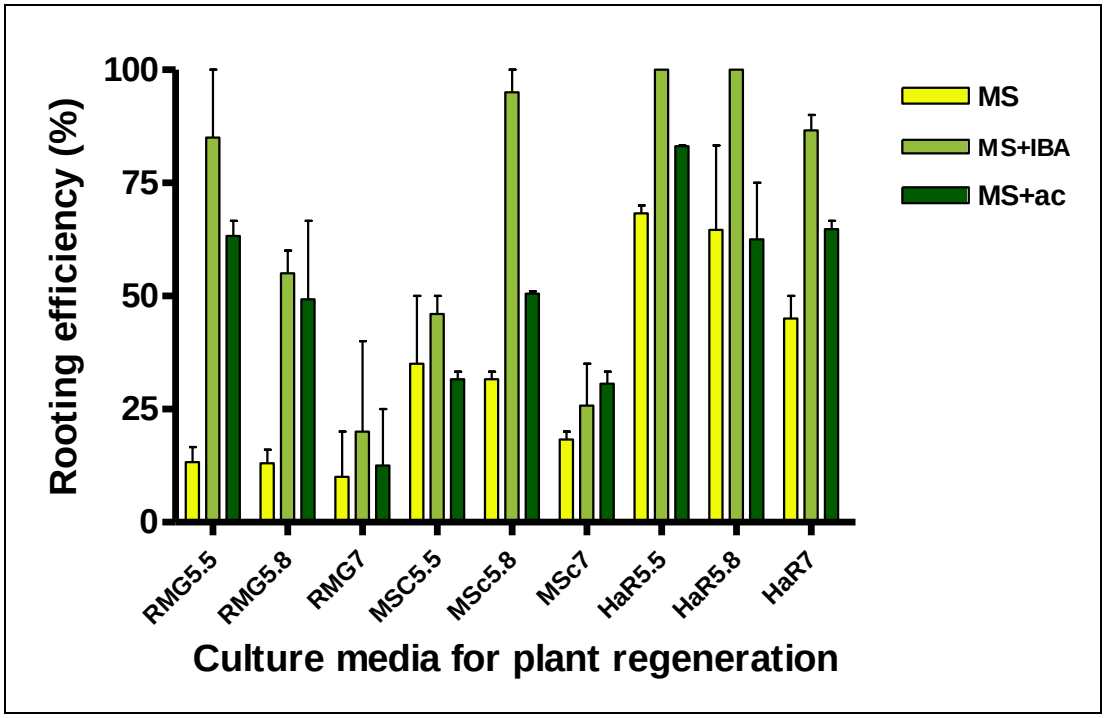

Fig. 10 The rooting efficiency of *in vitro* regenerated plants in sunflower, Turbo hybrid, on different rooting media (MS, MS+IBA, MS+ac)

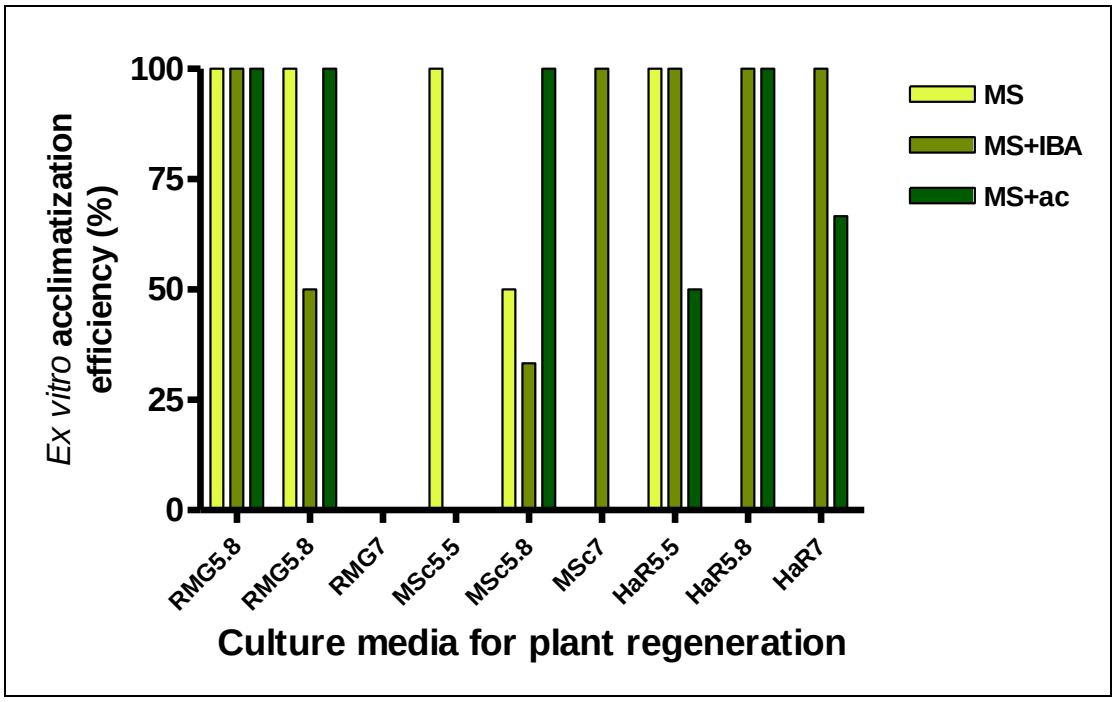

Fig. 11 The efficiency of *ex vitro* acclimatization of rooted plants in sunflower, Turbo hybrid

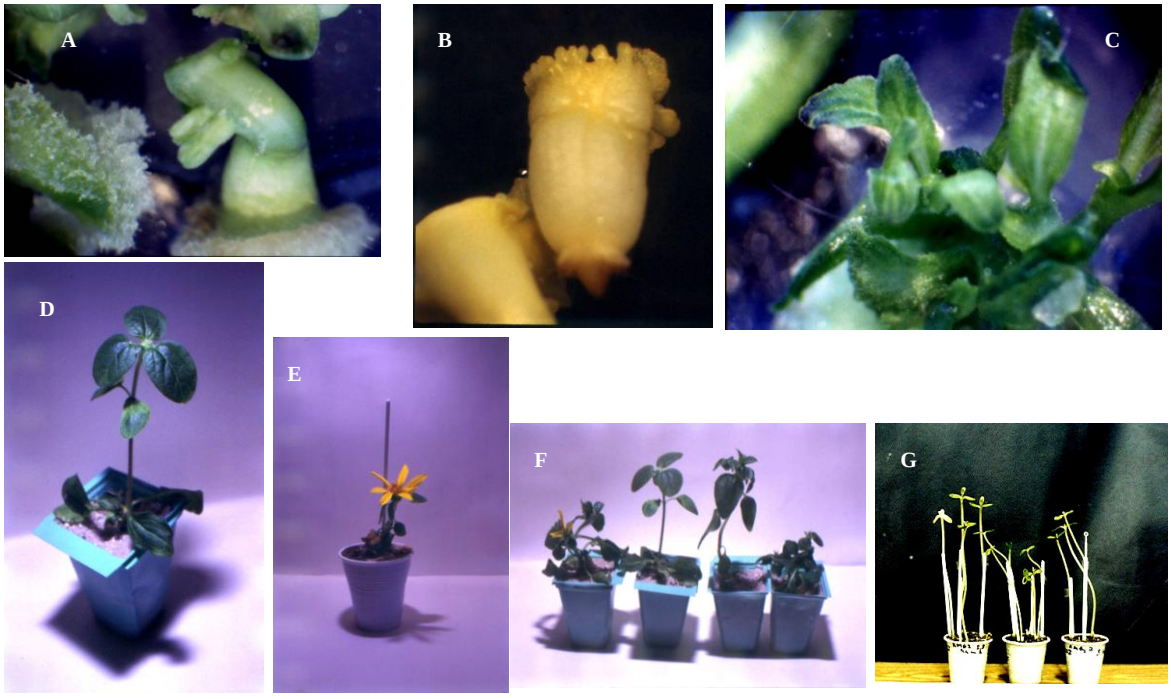

Fig. 12 Different stages of plant regeneration starting from mature embryonic axis in sunflower, Turbo hybrid: A, B – initial stage of shoot regeneration; C – one pare leaf stage of the regenerated shoots; D, E, F – well developed acclimated plants; G- plantlet resulted from seeds obtained from *in vitro* regenerated plants

3.4. The role of auxin on induction of caulogenesis versus embryogenesis in *in vitro* sunflower embryonic meristematic dome

Immature zygotic embryos often have a high morphogenetic potential and are frequently used for plant regeneration in species where regeneration from other types of tissues is difficult (Charriere and Hahne, 1998). The direct regeneration system for *in vitro* cultured immature zygotic embryos of sunflower is particular in that it is possible to experimentally orientate the regeneration response to either organogenesis or somatic embryogenesis, both events originating from an identical and well characterized cell population situated in the crown (Charriere and Hahne, 1998). As we previously mentioned the great disadvantage of this biologic material is the difficulty of obtaining it. Therefore the necessity of finding a regeneration method based on a starting tissue which is easy to be obtained all year round can be considered as particularly important.

The role of the auxin 2.4-D, dicamba, picloram, NAA and IAA were tested for induction of somatic embryogenesis in immature zygotic embryo explants. Only two of them, dicamba and 2,4-D, were optimal for somatic embryo initiation. No somatic embryos were formed when mature embryo or seedling tissues were placed on a medium which induced somatic embryogenesis from immature zygotic embryos (Finer, 1987).

Having all these aspects into consideration our goal was to establish a successful *in vitro* regeneration method starting from embryonic meristematic dome, a small explant having about 200-300 µm, resulted from mature ungerminated embryo (Fig. 13). A wide range of auxins IAA, IBA, NAA, 2.4-D, dicamba and picloram were tested in culture, in inducing medium at different concentrations. The culture medium that was used was MA1 (Rakosy-Tican, 1998) modified for nitrogen source and containing 3% sucrose.

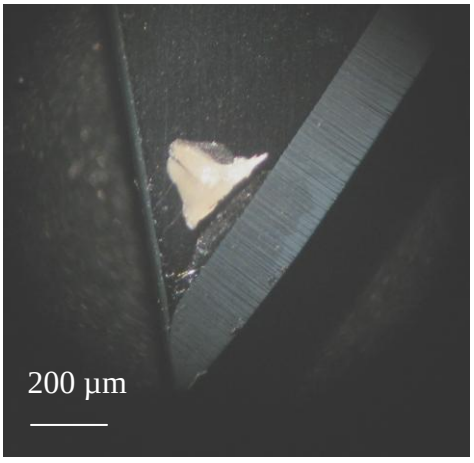

Fig. 13 Embryonic meristematic dome represented by apical meristem and primary leaf

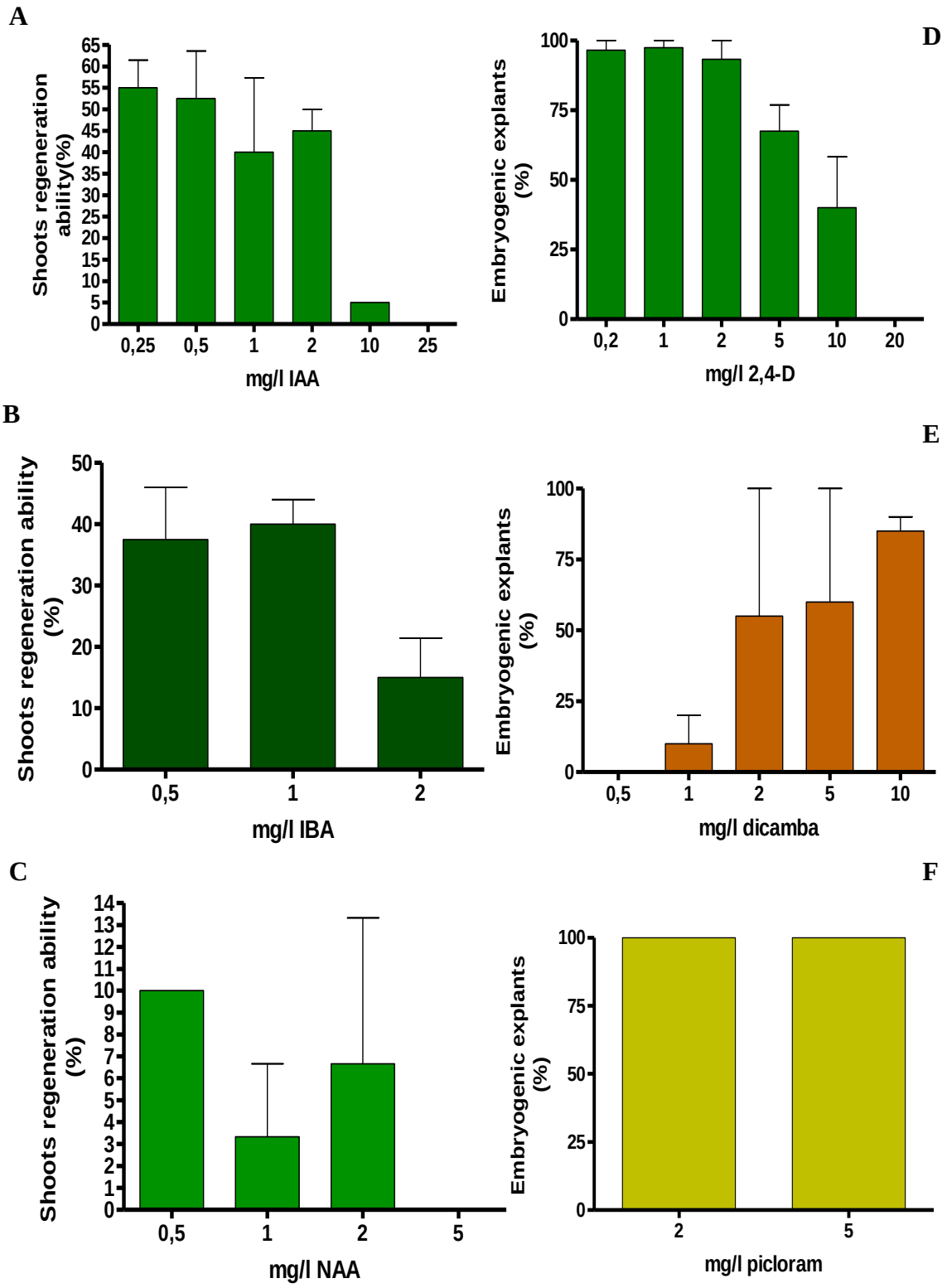

Fig. 14 Shoots regeneration efficiency in sunflower embryonic meristematic dome, Florina hybrid, on the culture medium with auxins IAA, IBA or NAA (A, B, C) and somatic embryo regeneration efficiency on the culture medium with auxins 2.4D, dicamba or picloram (D, E, F)

The response of embryonic meristematic dome was different depending on the type of the auxin from the culture medium. By applying the auxins IAA, IBA or NAA the regeneration occurred via organogenesis (Fig. 14 A, B, C). Contrary, by using the auxins 2.4-D, dicamba or picloram somatic embryos were obtained (Fig. 14 D, E, F). Among the organogenic auxins 0.25

or 0.5 mg/l IAA had the best response, up to 55% of the explant regenerating shoots. Surprisingly among the embryogenic auxins picloram had shown the best response, 100% of explants regenerating embryos. The auxins 2.4-D and dicamba induced, on the initiation medium, the regeneration of somatic embryos in globular and torpedo stages (Fig. 16). Only the picloram sustained the maturation of the embryos on the initiation medium containing in the same time 3% sucrose (Fig. 17).

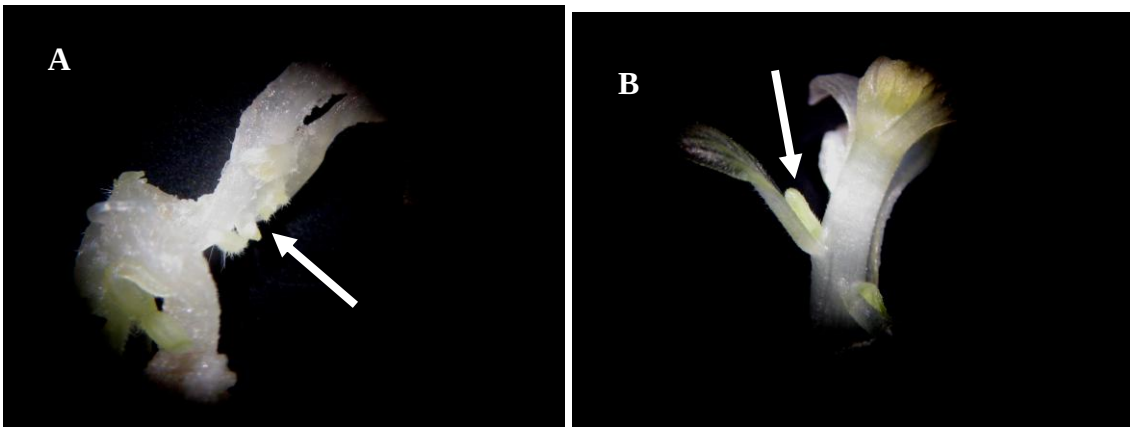

Fig. 15 Shoots regenerated in sunflower embryonic meristematic dome, Florina hybrid, on MA1v8 culture medium with 0..25 mg/l IAA (A) or 1 mg/l IAA (B) after 4 weeks; the shoots are regenerated on different points on the explant level – on the stem of the apical plant (A) axillary buds (B)

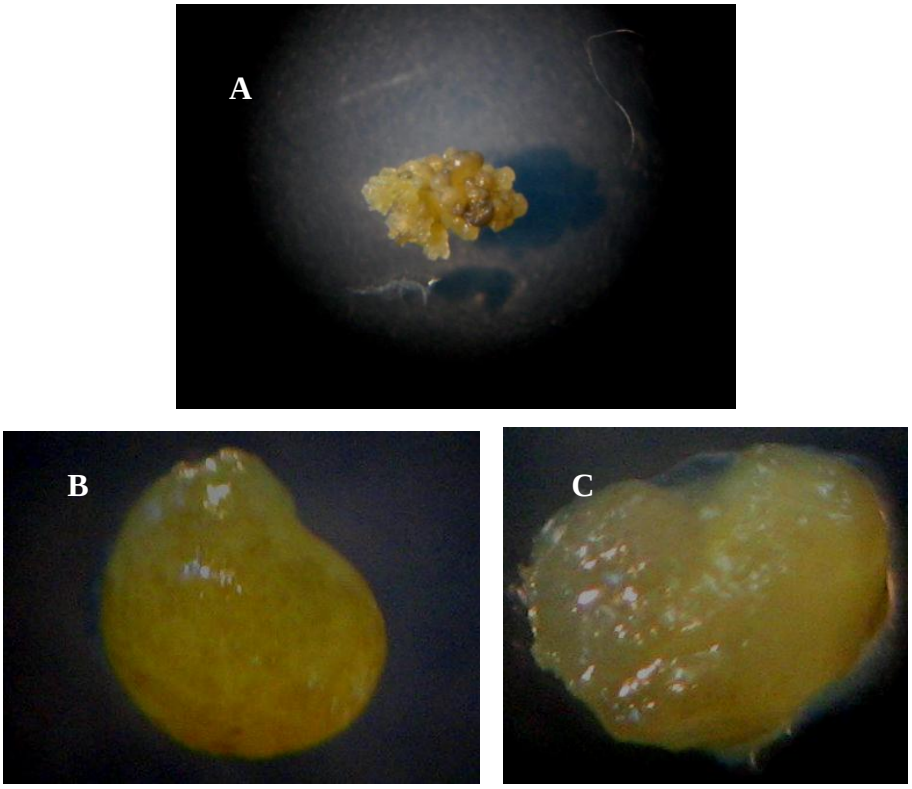

Fig. 16 Initial development stages of the somatic embryos regenerated in sunflower embryonic meristematic dome, Florina hybrid, after two weeks in culture on MA1v8 medium with 5 mg/L 2.4-D (A); somatic embryo in advanced globular stage - detail (B); heart shape stage of the somatic embryo (C)

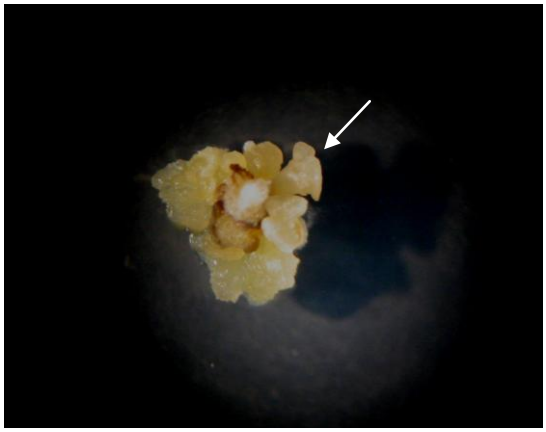

Fig. 17 Mature somatic embryos regenerated in sunflower, Florina hybrid, on MA1v8 culture medium with 2 mg/l picloram and 3% sucrose, after 4 weeks since the *in vitro* culture was initiated

To our knowledge, the embryonic meristematic dome resulted from mature ungerminated embryos has not been previously reported for sunflower regeneration. This kind of explant, consisting exclusively in meristematic cells, offers the opportunity of orienting the regeneration in either the direction of organogenesis or somatic embryogenesis, depending on the auxin type. Moreover this is the first report that proves the efficiency of picloram in regenerating mature somatic embryos in sunflower.

3.5. The role of sucrose in induction and maturation of sunflower somatic embryos, Florina hybrid

In this study an attempt to optimize the induction and maturation of somatic embryos by raising the concentration of sucrose in the culture medium. The regeneration method was described in the previous section. Several sucrose concentrations (3%, 6%, 9%. 12%) were added to the culture medium in order to improve somatic embryo development. In this case two factors had influence on the morphogenic response: the auxin type and the concentration of sucrose.

The best response was obtained on the medium containing 12% sucrose. Although the percentage of the explants that regenerate embryos remain unchanged for different sucrose concentrations the number of the somatic embryos on the explants is increasing in the case of using 12% sucrose. Therefore all the experiments were conducted further on 12% sucrose in the medium and compared with the control (3% sucrose).

Mature somatic embryos were regenerated on the induction medium when 12% sucrose was used in combination with 2.4-D, dicamba or picloram. By contrast to the situation in which the mature somatic embryo were obtained on media containing 3% sucrose and picloram, when they had a translucent white colour and thin cotyledons, on the medium with 12% sucrose all the mature somatic embryo presented a compact aspect of the cotyledons (Fig. 18, Fig. 19, Fig. 20).

Fig. 18 Mature somatic embryos regenerated in sunflower, Florina hybrid, on culture medium with 1 mg/l 2.4-D and 12% sucrose, after 4 weeks since the *in vitro* culture was initiated

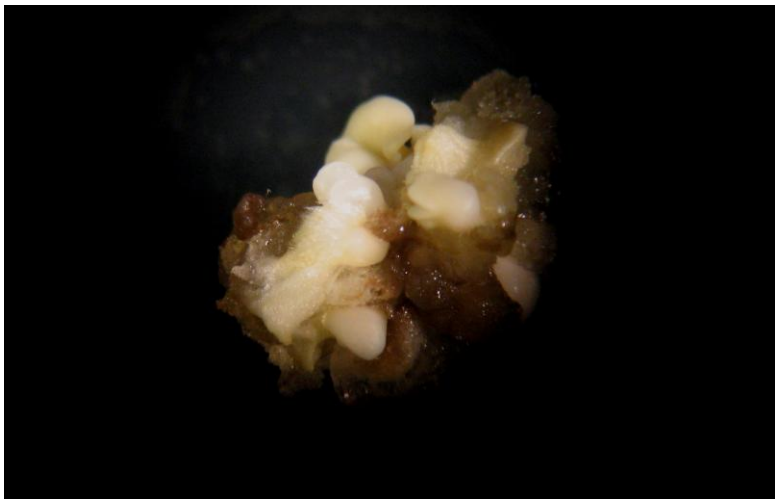

Fig. 19 Mature somatic embryos regenerated in sunflower, Florina hybrid, on culture medium with 0.5 mg/l picloram and 12% sucrose

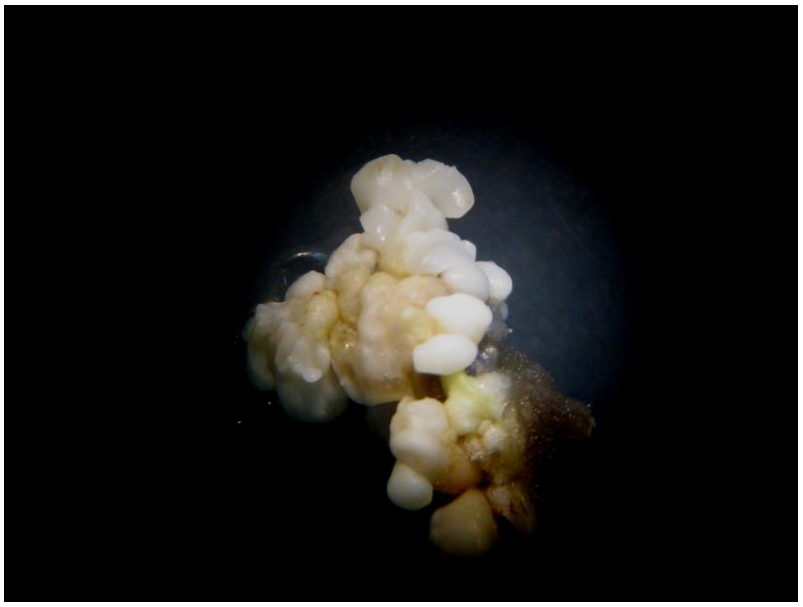

Fig. 20 Mature somatic embryos regenerated in sunflower, Florina hybrid, on culture medium with 2 mg/l dicamba and 12% sucrose

Somatic embryos were obtained in sunflower starting from immature zygotic embryos on medium with 12% sucrose and 2,4-D or dicamba (Finer 1987). In some cases it was proved that somatic embryogenesis ca be induced on medium containing 12% sucrose and no auxins (Jeannin et al., 1995). In other case the maturation of the somatic embryos was achieved in two steps: in the first the embryos were induced on medium with 3% sucrose and in the second one they developed up to maturity on a medium containing 6% sucrose (Wilcox McCann et al., 1988).

To our knowledge this is the first report of a one step method for regenerating mature somatic embryos in sunflower starting from embryonic meristematic dome by using different types of auxin and 12% sucrose in the culture medium. The usefulness of picloram on somatic embryo induction in an efficient manner was also emphasized.

3.6. The morphology of *in vitro* regenerated somatic embryos in sunflower and the relation with the embryo conversion

Abnormal morphological phenotypes have frequently been observed in *in vitro* regeneration of sunflower plants (Hewezi et al., 2003). The morphology of the somatic embryo wasn't discussed until now for sunflower. Abnormalities in somatic embryo and cotyledon development have been observed for other species such are peanut (Wetzstein and Baker, 1993), soybean (Buchheim et al., 1989) and apple (Paul et al., 1994).

The most frequent abnormalities observed in this study were similar to those described in soybean (Buchheim et al., 1989) i.e. trumpet types resulted by fusion of the cotyledons, those with long hypocotyls, vestigial cotyledons, monocotyledonous, or fasciated. The majority resulted when the auxins NAA and 2.4-D were presented in the culture medium or when 2.4-D was used as sole auxin.

The abnormalities of somatic embryos are often related with impossibility of their further conversion. In this study the embryos cultured on media without growth regulators rooted well but were deficient for apical meristem development.

In order to improve the recovery of plants from somatic embryos we included in the regeneration medium an amino acid compound – 500 mg/L tryptone. Although the embryos are grouped in clusters, they were able to develop both meristems (Fig. 21).

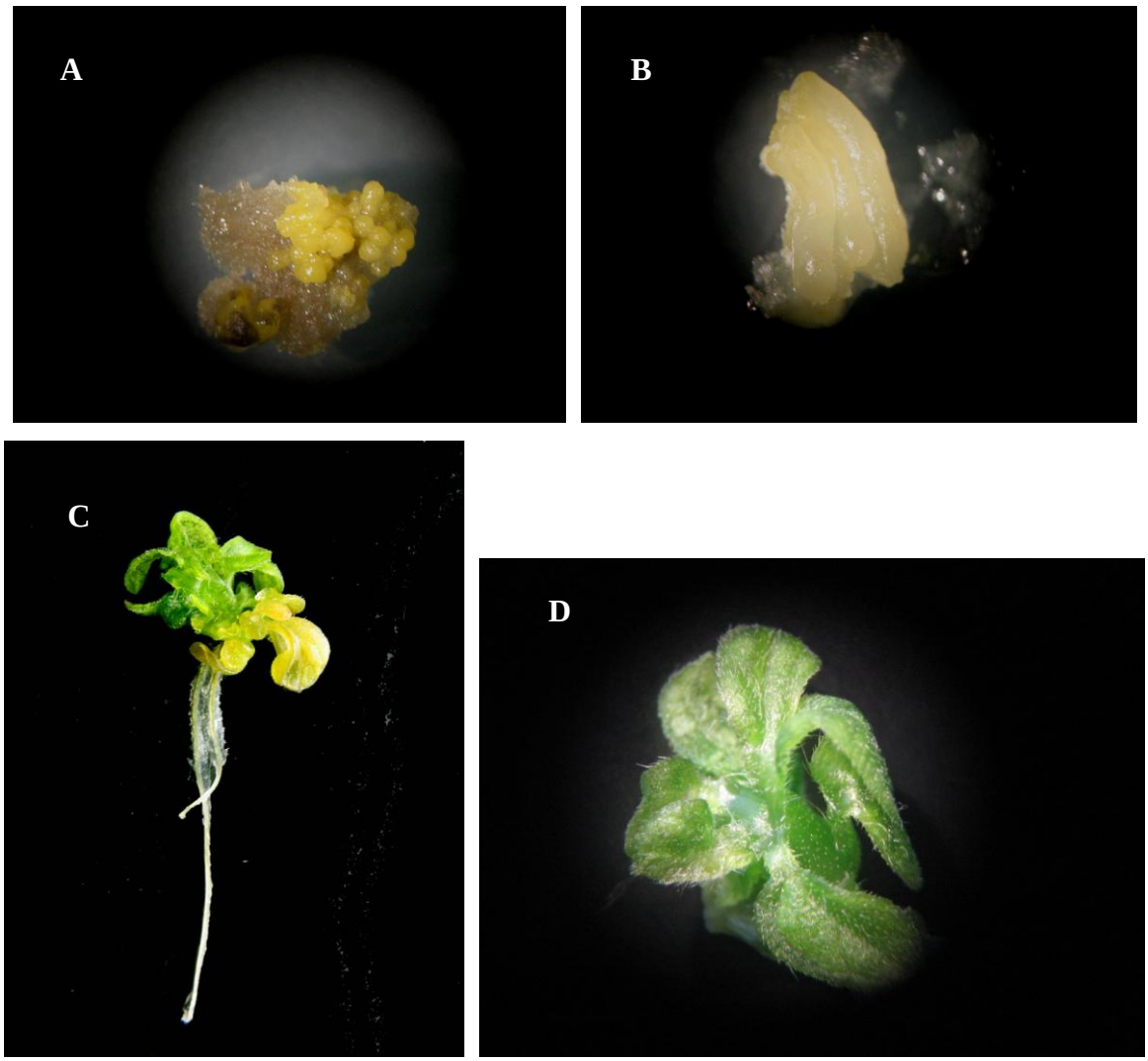

Fig. 21 Somatic embryos in globular stage (A) and grouped somatic embryos in mature stage (B), regenerated on MA1 medium with tryptone and germinated on RMB5 medium (C); regenerated plant – detail (D)

3.7. The role of haemoglobin on protoplast plating efficiency and cell colony formation in sunflower, Select hybrid

Novel approaches, involving supplementation of aqueous culture medium with haemoglobin solution have been evaluated to facilitate cellular oxygen availability to promote mitotic division (Anthony et al., 1997b). This compound was used in protoplast or cell culture for different species (Anthony et al., 1997a; Anthony et al., 1997b).

The goal of our study was to stimulate protoplast plating efficiency and the cell colony formation by adding to the culture medium the 1:50 (vol:vol) haemoglobin (Erytrogen). Protoplasts isolated from hypocotyls were embedded in an alginate disc and immersed in liquid culture medium.

The protoplast plating efficiency was evaluated at 4 and 20 days of culture. The mean initial protoplast plating efficiency after 4 days with haemoglobin was significantly higher

(6.41±1.11%) than in untreated control (2.63±0.63%). The final plating efficiency at 20 days had the same pattern, a two–fold increase of the mean value on haemoglobin (30.79±1.93 %) as compared to the control (14.97±1.63 %).

The beneficial role of haemoglobin was further reflected in the mean number of protoplast-derived cell colonies visible by naked eye after 40 days in culture (87.56±19.8%) compared with the control, 52±21.05 %. Considering three independent experiments the total number of cell colonies obtained on culture medium supplemented with haemoglobin vas 787 comparing with 424 obtained in the control (Fig. 22).

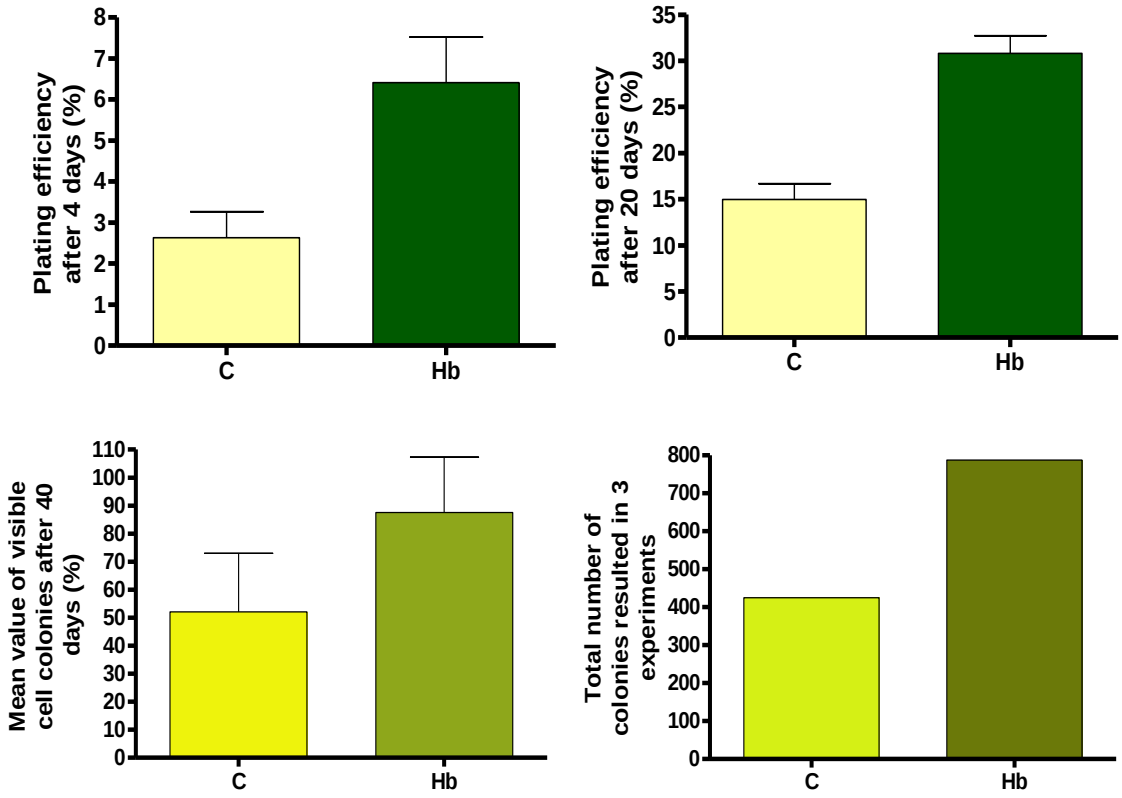

Fig. 22 The initial (at 4 days) and final (at 20 days) protoplast plating efficiency and the cell colony recovery in sunflower with or without 1:50 (vol:vol) haemoglobin in the liquid culture medium

Further studies are required to determine the precise mechanism by which haemoglobin can facilitate the protoplasts and the cells division. The present data imply that the commercial Erythrogen could be incorporated routinely into culture medium, in order to increase protoplast - derived cell colonies regeneration.

3.8. Enhancing transient expression of *gfp* gene by wounding of intact embryonic axis prior to *Arobacterium* infection

Preliminary investigations regarding the best ways to improve genetic transformation of sunflower by using *Agrobacterium tumefaciens* carrying the reporter gene *gfp* were performed. This gene wasn't used until now for genetic transformation of mature sunflower tissues. Previously, only immature zygotic embryos were transformed with *gfp* gene (Müler şi colab., 2001).

Several wounding methods were approached in this study in order to improve genetic transformation efficiency of sunflower by using mature embryonic axis as starting material, for Turbo and Florina hybrids. One of them consisted in the simple scratching with a scalpel blade of the embryonic axis prior to *Agrobacterium* treatment. The superficial wounding of the explants significantly increased the transient expression of *gfp* in sunflower tissues, up to 94% of the explants manifesting the green fluorescence in UV light compared to 70% in unwounded controls (Fig. 23).

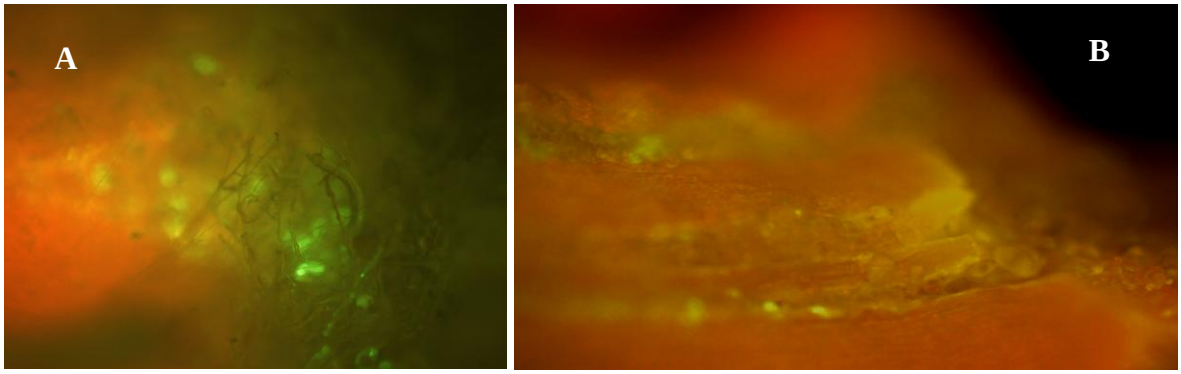

Fig. 23 Areas of *gpf* expresion in trangenic tissue (in the UV light) - the root zonne (A), or the wounded zone obtined by scratching with a scalpel blade of the embryonic axis (B)

Another way for wounding of the tissues was the superficial enzymatic digestion of the mature embryonic axis for about 4 ours, using a solution of 0.1% or 0.2% macerozyme. After enzymatic digestion the explants were treated with *Agrobacterium*. Among the two enzyme concentrations which were tested only the 0.2% value was efficient for enhancing the transient expression of the *gfp* (Fig. 25).

In order to improve transformation efficiency the explants were subjected to electrical pulses during the *Agrobacterium* treatment. Among different parameters of the electric pulses the most efficient were those having a voltage of 200 V/cm and duration of 400 µs (Fig. 24).

Although the manifestation of *gfp* in tissues was transient and none of the regenerated plants showed transgenic expression, the method presents the great advantage of direct

visualisation of the green fluorescence in tissues thus permitting further optimisation of the protocol.

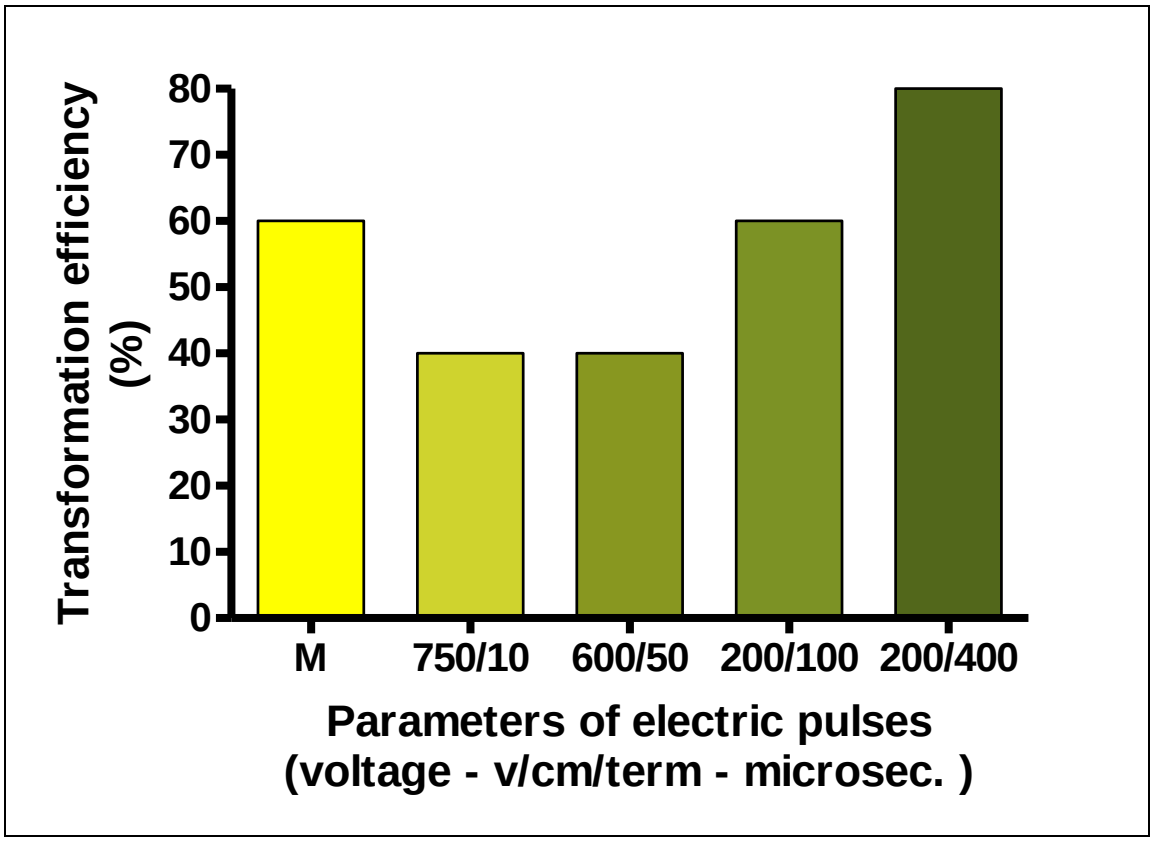

Fig. 24 The efficiency of transient *gfp* expression in mature embryo axis as resulted by applying of two electric pulses during the *Agrobacterium* treatment

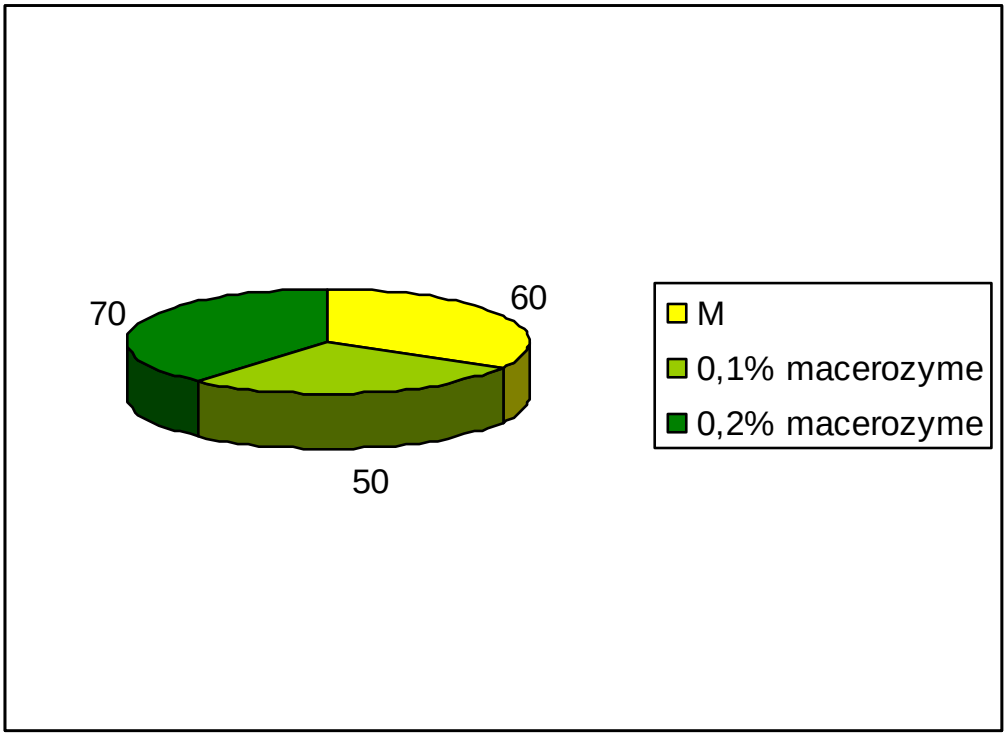

Fig. 25 The efficiency of transient *gfp* expression in mature embryo axis as resulted by enzymatic digestion with macerozyme of the explants prior to *Agrobacterium* treatment

4. GENERAL CONCLUSIONS

The first study involves, in an extended way, the comparison between age dependant embryonic axis regenerating potential and age dependant cotyledon fragments regenerating potential. The explant age was equally critical for cotyledon explants and for embryonic axis upon regeneration. The regenerative potential of the embryonic axis is higher when they resulted from ungerminated mature embryos compared whit the cotyledon explants who respond better when they resulted from two days old germinated embryos. Between the two types of explants the most efficient for plant regeneration was the embryonic axis.

By comparing 6 types of explants resulted from ungerminated mature embryos it was proved once more that the intact embryonic axis explant has high regeneration potential in sunflower.

By using the intact embryonic axis resulted from ungerminated mature embryos, an efficient regeneration method was established for *in vitro* plant regeneration at 14 genotypes: thirteen hybrids and one line. The fertile plant regeneration was achieved for Turbo hybrid. The presence of IBA and pH 5.5 were found suitable for the efficient rooting of the regenerated plants.

A new regeneration method was found by starting from embryonic meristematic dome. During the experimental testing of the auxins IAA, IBA, NAA, 2.4-D, dicamba and picloram, it was demonstrated that three of them had organogenic potential (IAA, IBA, NAA) and the others had embryogenic potential (2.4-D, dicamba and picloram). The picloram proved to be efficient for mature embryo regeneration on the induction medium.

Using the sucrose in high concentration (12%) the maturation of the embryos was achieved on the induction medium for all the embryonic auxins (2.4-D, dicamba and picloram). The abnormalities of the embryos were correlated with deficiencies of apical meristem development on the medium without growth regulators. Using tryptone in the induction medium the conversion of the embryos was possible.

The stimulatory effect of haemoglobin was proved by its ability to enhance the protoplast plating efficiency and protoplast-derived cell colony regeneration in sunflower.

The embryonic axis resulted from mature ungerminated embryos was used for *Agrobacterium* – mediated transformation. The transient *gfp* expression was obtained at callus stage. Different wounding ways of the embryonic axis can enhance the transformation efficiency.

Original elements

- a regeneration method for several hybrids of sunflower was established;
- the high regeneration ability of the sunflower embryonic meristematic dome by organogenesis was induced, in the presence of the auxins IAA, IBA, NAA, or somatic embryogenesis, in the presence of the auxins 2.4-D, dicamba or picloram;
- the role of picloram in inducing mature somatic embryo regeneration to sunflower was demonstrated;
- the stimulatory effect of haemoglobin was demonstrated in sunflower protoplasts culture.

Recommendation and perspective

The embryonic meristematic dome can be considered a useful model system for the study of morphogenesis in plants because it's high regeneration response and high plasticity of regeneration. The type of regeneration can be easily oriented towards organogenesis or somatic embryogenesis by modifying a single parameter, the auxin type.

The usefulness of the reporter gene *gfp* in transgenic tissue was demonstrated once more in our study. Because it's easy detection in tissues it permits a direct evaluation of the transformation efficiency and, if it's necessary, subsequent improvements of the method of recovery transgenic plant.

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REFERENCES (SELECTION):

1. **Alibert G., Aslane-Chanabe C., Burrus M. 1994** – Sunflower tissue and cell culture and their use in biotechnology. Plant Physiol. Biochem., 32: 31-44.
2. **Anthony P., Lowe K. C., Davey M. R., Power J. B. 1997 b –** Strategies for promoting division of cultured plant protoplasts: synergistic beneficial effects of haemoglobin (Erythrogen) and Pluronic F-68. Plant Cell Rep., 17: 13-16.
3. **Aurori A., Szmolka A., Rakosy-Tican L. 2000 -** *In vitro* regeneration and genetic transformation of sunflower (*Helianthus annuus* L.) In: Craciun C. si Ardelean A. (eds) Current Problems in Cellular and Molecular Biology, papers of The First International Congress of the Romanian Society for Cell Biology Iasi, Risoprint, Cluj-Napoca, pp. 543-546.
4. **Baker C. M., Munoz-Fernandez N., Carter C. D. 1999** – Improved shoot development and rooting from mature cotyledons of sunflower. Plant Cell Tissue Organ Cult. 58: 39-49.
5. **Buchheim J. A., Colburn S. M., Ranch J. P. 1989** – Maturation of soybean somatic embryos and the transition to plantlet growth. Plant Physiol., 89: 768-775.
6. **Charriere F., Hahne G. 1998** – Induction of embryogenesis versus caulogenesis on *in vitro* cultured sunflower (*Helianthus annuus* L.) immature zygotic embryos: role of plant growth regulators. Plant Sci., 137: 63-71.
7. **Christianson M. L., Warnick D. A. 1984.** Phenocritical times in the process of *in vitro* shoot organogenesis. Dev. Biol., 101: 382-390.
8. **Deglene L., Lesignes P., Alibert G., Sarrafi A. 1997** – Genetic control of organogenesis in cotyledons of sunflower (*Helianthus annuus*). Plant Cell Tissue Organ Cult. 48: 127-130.
9. **Espinasse A., Lay C.** 1989 – Shoot regeneration of callus derived from globular to torpedo embryos from 59 sunflower genotypes. Crop Sci., 29: 201-205.
10. **Fauguel C. M., Vega T. A., Nestartes G., Zorzoli R., Picardi L. A. 2008** – Anatomy of normal and hyperhydric sunflower shoots regenerated *in vitro*. Helia, 31: 17-26.
11. **Fernandez-Martinez J. M., Perez-Vich B., Velaso L., Dominguez J. 2007** – Breeding for specialty oil types in sunflower. Helia, 30: 75-84.
12. **Fiore M. C., Trabace T., Sunseri F. 1997** – High frequency of plant regeneration in sunflower from cotyledons *via* somatic embryogenesis. Plant Cell Rep. – Abstr., 16: 295-298.

13. **Finer J. J. 1987** - Direct somatic embryogenesis and plant regeneration from immature embryos of hybrid sunflower (*Helianthus annuus* L.) on high sucrose- containing medium. Plant Cell Rep., 6: 372-374.
14. **Freyssinet M., Freyssinet G. 1988** - Fertile plant regeneration from sunflower (*Helianthus annuus* L.) immature embryos. Plant Sci., 56: 177-181
15. **Hewezi T., Jardinaud F., Alibert J., Kallerhoff J. 2003** - A new approach for efficient regeneration of a recalcitrant genotype of sunflower (*Helianthus annuus* L.) by organogenesis induction on split embryonic axes. Plant Cell Tissue Organ Cult., 00: 1-6.
16. **Honda Y., Mukasa Y., Suzuk T. 2005** – Traits of NuSun^{TM} varieties of sunflower in Hokkaido, Japan. Plant Prod. Sci., 8: 461-464.
17. **Jeannin G., Bronner R., Hahne G. 1995** - Somatic embryogenesis and organogenesis induced on the immatutre zygotic embryo of sunflower (*Helianthus annuus* L.) cultivated *in vitro*: role of the sugar. Plant Cell Rep., 15: 200-2004.
18. **Knittel N., Escandon A. S., Hahne G. 1991** – Plant regeneration at high frequency from mature sunflower cotyledons. Plant Sci. 73: 219-226.
19. **Molinier J., Himber C., Hahne G. 2000** - Use of green fluorescent protein for detection of transformed shoots and homozygous offspring. Plant Cell Rep., 19: 219-223.
20. **Murashige T. and Skoog F. 1962** - A revised medium for growth and rapid bioassys with tobacco culture. Phisiol. Plant., 15: 473-497.
21. **Müller A., Iser M., Hess D. 2001** – Stable transformation of sunflower (*Helianthus annuus* L.) using a non-meristematic regeneration protocol and green fluorescent protein as a vital marker. Transgenic Res., 10: 435-444.
22. **Paterson K. E. and Everett N. P. 1985** - Regeneration of *Helianthus annuus* inbred plants from callus. Plant Science, 42: 125-132.
23. **Paul H., Belaizi M., Sangwan-Norreel B. S. 1994** – Somatic embryogenesis in apple. J. Plant Physiol., 143: 78-86.
24. **Power C. J. 1987** – Organogenesis from *Helianthus annuus* inbreds and hybrids from the cotyledons of zygotic embryos. Amer. J. Bot., 74: 497-503.
25. **Pugliesi C., Megale P., Cecconi F., Baroncelli S. 1993** – Organogenesis and embryogenesis in *Helianthus tuberosus* and in the interspecific hybrid *H. annuus x H. Tuberosus*. Plant Cell Tissue Organ Cult., 33: 187-193.
26. **Rakosy-Tican L. 1998** - Utilizarea Tehnicilor de Electrofuziune în Hibridarea Somatică a Plantelor. Presa Universitară Clujeană, Cluj-Napoca.
27. **Rakosy-Tican L., Aurori A., Aurori C. 2000** – Green fluorescent protein (GFP) – a new marker gene for plant genetic engineering. In: Craciun C. si Ardelean A. (eds) Current Problems in Cellular and Molecular Biology, papers of The First International

Congress of the Romanian Society for Cell Biology Iasi, Risoprint, Cluj-Napoca, pp. 532-537.

28. **Sujatha M., Prabakaran A. J.** 2001 **–** High frequency embryogenesis in immature zygotic embryos of sunflower. Plant Cell, Tissue, Organ Cult., 65: 23-29.
29. **Vrânceanu A. V. 2000** – Floarea Soarelui Hibridă. Ed. Ceres, Bucureşti
30. **Wetzstein H. Y., Baker C. M. 1993** - The relationship between somatic embryo morphology and conversion in peanut (*Arachis hypogaea* L.). Plant Sci., 92: 81-89
31. **Wilcox McCann A., Cooley G., Van Dreser J. 1988** - A system for routine plantlet regeneration of sunflower (*Helianthus annuus* L.) from immature embryo-derived callus. Plant Cell Tissue Organ Cult., 14: 103-110
32. **Witrzens B., Scowcroft W. R., Downes R. W., Larkin P. J. 1988** - Tissue culture and plant regeneration from sunflower (*Helianthus annuus*) and interspecific hybrids (*H. tuberosus* x *H. annuus*). Plant Cell Tissue Organ Cult., 13: 61-76.

LISTA DE LUCRĂRI

1. Papers in peer-reviewed journals

Aurori A., Rakosy-Tican L., Ghenescu Vesa S., 2004. Cercetări de inginerie genetică la floarea-soarelui. Cercetări de Genetică Vegetală şi Animală (VIII), 45-55.

2. Papers in volumes of conference

Aurori A., Rakosy-Tican E. 2005. Inducerea embriogenezei somatice şi a caulogenezei în cultura apexului embrionar la floarea-soarelui, hibridul Florina. În: Conservarea Vitroculturilor Vegetale – al XIV-lea Simpozion Național de Culturi de Țesuturi şi Celule Vegetale, D. Cachiță-Cosma şi C. Sand (Eds), Ed. Alma Mater Sibiu, 192-206.

Aurori A., Ursu T., Rakosy-Tican L. 2002. Rezultate preliminarii privind optimizarea culturii *in vitro* a explantelor tisulare de floarea-soarelui (*Helianthus annuus* L.) hibridul Turbo. În: Lucrările celui de-al X-lea Simpozion Național de Culturi de Țesuturi şi Celule Vegetale, D. Cachiță-Cosma, L. Rakosy-Tican, A. Ardelean (Eds.), Ed. Risoprint, Cluj-Napoca, 279-286.

Rakosy-Tican L., Aurori C. M., **Aurori A**. 2002. Transformarea genetică – de la genele marker la gene de interes economic. În: Lucrările celui de-al X-lea Simpozion Național de Culturi de Țesuturi şi Celule Vegetale, D. Cachiță-Cosma, L. Rakosy-Tican, A. Ardelean (Eds.), Ed. Risoprint, Cluj-Napoca, 147-278.

Aurori A., Szmolka A., Rakosy-Tican L. 2000. *In vitro* regeneration and genetic transformation of sunflower (*Helianthus annuus* L.). În: Curent Problems in Cellular and Molecular Biology (V), C. Crăciun şi A. Ardelean (Eds.), Ed. Risoprint Cluj-Napoca, 543-546.

Rakosy-Tican L., **Aurori A.**, Aurori C. M. 2000. Green fluorescent protein (GFP) – a new marker gene for plant genetic engineering. În: Curent Problems in Cellular and Molecular Biology (V), C. Crăciun şi A. Ardelean (Eds.), Ed. Risoprint Cluj-Napoca, 532-537.

Rakosy-Tican L., Ispas G., Biro J., Aurori C. M., **Aurori A**. 1999. Realizări recente în domeniul ingineriei genetice vegetale. În: Lucrările Simpozionului "Prezent şi Perspectivă în Horticultură", 21-23 Decembrie, Cluj-Napoca, 9-16.

Aurori A., Rakosy-Tican L., Kreibik Ş. 1999. Studii preliminare asupra electrostimulării calusului de floarea-soarelui. În: Culturi *In Vitro* la Cormofite, D. Cachiță-Cosma, A. Ardelean, C. Crăciun (Eds.). Ed. Risoprint Cluj-Napoca, 292-296.

Aurori A., Rakosy-Tican L., Comşa D., Kreibik Ş., Popescu Ş. 1999. The effect of electric field on sunflower (*Helianthus annuus*) protoplasts and protoplast derived callus. În: Curent Problems in Cellular and Molecular Biology (IV), A. Ardelean şi C. Crăciun (Eds.), Ed. Risoprint Cluj-Napoca, 637-641.

Aurori A., Rakosy-Tican L., Morariu V. 1998. Searching for new ways to stimulate sunflower protoplast regeneration. În: Curent Problems in Cellular and Molecular Biology (III), C. Crăciun şi A. Ardelean (Eds.), Ed. Risoprint Cluj-Napoca, 497-50

3. Papers in peer-reviewed international journals

Rakosy-Tican E., **Aurori A.**, Vesa S., Kovacs K-M. 2007. *In vitro* morphogenesis of sunflower (*Helianthus annuus*) hipocotil protoplasts: the effects of protoplasts density, haemoglobin and spermidine. Plant Cell Tiss. Organ Cult., 90: 55-62.

4. Posters or presentations at conferences

Aurori A., Rakosy-Tican E. 2006. *In vitro* culture of embryonic apex as a model system for sunflower plant regeneration. In abstracts book of Seventh European Conference on Sunflower Biotechnology, September 3-6, Gengenbach, Germany, pg 33 (abstract)

Rakosy-Tican E., **Aurori A**., Ursu T. 2006. Fertile plant regeneration through three step culture procedure from mature embryo axis in sunflower cultivars and transient expression of green fluorescent protein reporter gene in mature embryo axis-derived callus. SUNBIO - Seventh European Conference on Sunflower Biotechnology, September 3-6, Gengenbach, Germany, pg. 25 (abstract)

Aurori A., Rakosy-Tican L., Ursu T., Ghenescu S. 2003. Improved *in vitro* shoot regeneration, rooting and *ex vitro* development from proximal mature seed explant of ten sunflower genotypes. SUNBIO – Sixt European Conference on Sunflower Biotechnology, 5-9 October, Seville, pg. 62 (abstract)

Ghenescu S., Rakosy-Tican L., **Aurori A.** 2002 - Influence of spermidine on callus regeneration and somatic embryo production from *Helianthus annuus* L. hypocotyls protoplasts. Forum for Young Scientists. 18-20 Octomber. Istanbul, Turkei. 68 (abstract)

Other publication

1) Elena Rakosy-Tican, **Adriana Aurori**, Camelia Dijkstra, Maria C. Maior (2010) Generating marker free transgenic potato cultivars with an hairpin construct of PVY coat protein. *Romanian Biotechnological Letters Vol. 15, No.1, Supplement:* 63-71 (ISI fara IF)
2) Aurori C.M., **Aurori A.**, Maior M, Coroian C., Dezmirean D., Marghitas L.A., Rakosy-Tican E. (2008) The analysis of stability in constitutively expressed transgenes *gfp* and *npt* II in tobacco, used as markers for possible horizontal gene transfer to honeybees and their microflora. 1st Global Conference on GMO Analysis, 24-27 June 2008, Como Italy Book of Abstracts, p. 113.
3) Rakosy-Tican E, Thieme R., **Aurori A.**, Aurori C.M., Rokka V-M. (2008). Combining marker assisted selection (MAS) and somatic hybridization for better introgression of resistance genes into cultivated potato. 17th Triennial Conference of the European Association for Potato Research (EAPR). Potato for a changing world. Abstracts of Papers and Posters. EAPR 2008 July 6-10 Brasov Romania, p. 83.
4) Aurori C.M., **Aurori A**., Rakosy-Tican E. (2008) *Agrobacterium*-mediated transformation of *S. bulbocastanum* and potato (*S. tuberosum* cv. Delikat) with msh2 deficient genes. 17th Triennial Conference of the European Association for Potato Research (EAPR). Potato for a changing world. Abstracts of Papers and Posters. EAPR 2008 July 6-10 Brasov Romania, p 421.
5) **Aurori A.**, Ispas G., De Riek J., Angenon G., Famelaer I., Rakosy-Tican E. (2008) Improoving crop genetic pool by somatic hybridization using DNA mismatch repair (MMR) deficient plants. 17th Triennial Conference of the European Association for Potato Research (EAPR). Potato for a changing world. Abstracts of Papers and Posters. EAPR 2008 July 6-10 Brasov Romania, p 422.
6) Maior M, **Aurori A.,** Rakosy-Tican E. (2008) Microtuberization as an efficient way for *in vitro* medium-term conservation of Solanum wild species, potato somatic hybrids or transgenic plants. 17th Triennial Conference of the European Association for Potato Research (EAPR). Potato for a changing world. Abstracts of Papers and Posters. EAPR 2008 July 6-10 Brasov Romania, p 423.
7) Elena Rakosy-Tican, Ramona Thieme, Marion Nachtigal, **Adriana Aurori**, Jan de Riek, Veli-Matti Rokka. 2008. The genotype of the recipient potato variety plays an important role

for the genetic makeup of the somatic hybrids of Solanum tuberosum and late blight resistant S. bulbocastanum. SolGenome – The 5th Solanaceae Genome Workshop, 12-16 oct. 2008, Cologne Germany, p.224.

8) Elena Rakosy-Tican, Maior C. M., **Aurori A**., Isac V. Cap.5. Selectia somaclonala a unor forme valoroase de trandafir. In. M. Palada-Nicolau (coord.), Biotehnologii de inmultire, selectie si conservare la plante ornamentale lemnoase. Academic Press Cluj-Napoca 2008 ISBN 978-973-744-122-5, p. 95-107.
9) Elena Rakosy-Tican, **Adriana Aurori**, Camelia Dijkstra, Cristian M. Aurori, Maria C. Maior, Ramona Thieme, Michael Davey. (2007). A Simple Strategy to Generate a Large Number of Marker-Free Transgenic Potato Plants. Plant Transformation Technologies, Vienna 4-7 February 2007 - Book of Abstracts, p. 88.
10) Elena Rakosy-Tican, Aurori C., **Aurori A**., Dijkstra C., Thieme R., Maior M., Davey M.R. (2007). Genetic transformation of potato dihaploid lines and tetraploid cultivars by using reporter gene gfp and PVY-CP genes. Workshop of the Pannonian Plant Biotechnology Association: "Plant Biotechnology and Stability of Crop Production in the Great Pannonian Region" Piešťany, Slovakia, April 17-19, 2007
11) Elena Rakosy-Tican, **Adriana Aurori**, Ramona Thieme, Thomas Thieme, Radu Grumeza, Ivan Famelaer, Jan De Riek, Geert Angenon. (2007). Somatic hybrids between potato and *Solanum chacoense* accessions highly resistant to Colorado potato beetle. EUCARPIA Plant genetic Resources and their Exploitation in the Plant breeding for Food and Agriculture, Piestany, Slovak republic 23-26 May, 2007 – Book of Abstracts, p.50 –
12) Aurori C.M., **Aurori A.,** Rakosy-Tican E. (2007) Tissue culture and somatic hybridization techniques – new sources for ornamental traits in *Solanum tuberosum* and *S. chacoense*. 5th International Conference "Propagation of Ornamental Plants" 5-8 September 2007, Sofia Bulgaria. Book of abstr., pp. 91.
13) Maior M., **Aurori A.**, Vesa S., Rakosy-Tican E. **(**2007). *In vitro* culture of Romanian rose cultivars. 5th International Conference "Propagation of Ornamental Plants" 5-8 September 2007, Sofia Bulgaria. Book of abstr., pp. 136.
14) Maior M., **Aurori A**., Vesa S., Wagner St., Rakosy-Tican E. 2007. Micropropagarea, multiplicarea şi înrădăcinarea *in vitro* a unor soiuri de trandafir. In: Cachiță-Cosma D. (coord.), Micropropagarea speciilor vegetale – Lucrările celui de al XV-lea Simpozion Național de Culturi de Tesuturi și Celule Vegetale, Iași 2006, Ed. Risoprint Cluj-Napoca, 2007, pp. 205-212.
15) Rakosy-Tican L., Aurori C.M., Dijkstra C., Thieme R., **Aurori A.,** Davey M.R. (2006) The usefulness of reporter gene *gfp* for monitoring A*grobacterium*-mediated transformation of potato dihaploid and tetraploid genotypes. Plant Cell Reports, 26:661-671, DOI: 10.1007/s00299-006-0273-8 (IF=**1,974**)
16) **Aurori A.,** Ispas G., De Riek J., Famelaer I., Angenon G., Rakosy-Tican E. (2006). Inducing mismatch repair system deficiency in *Solanum chacoense* and its usefulness for somatic hybridization with *Solanum tuberosum*. XV FESPB Congress, 17-21 July Lyon France, Book of abstracts p. 63 (prezentare – 15 min)
17) Rakosy-Tican L., Aurori C.M., **Aurori A,** Busuioc A., Ispas G., Antonova O., Famelaer I. 2005. Agrobacterium tumefaciens mediated transformation of Solanum chacoense Bitt. – the use of reporter gene gfp and MSH2 genes. Romanian Journal of Genetics 1 (1): 74-83.
18) Rakosy-Tican E., **Aurori A,** Thieme R., Grumeza R., Famelaer I., De Riek J., Angenon G. 2005. Cytogenetic and molecular characterization of somatic hybrids between Solanum cultivars and Solanum bulbocastanum. Romanian Journal of Genetics 1 (2): 58-67.
19) Rakosy-Tican L**., Aurori A**., Aurori C.M., Ispas G., Famelaer I. 2004. Transformation of wild *Solanum* species resistant to late blight by using reporter gene *gfp* and *msh2* genes. *Plant Breeding and Seed Science* (Warszawa), 50: 119-128.