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High frequency somatic embryogenesis and plant regeneration from zygotic embryo-derived callus cultures of three *Allium* species

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Abstract

The plant regeneration ability of zygotic embryo-derived callus cultures was studied for 12 *A. cepa* varieties and accessions, two *A. fistulosum* varieties, one *A. fistulosum* × *A. cepa* interspecific hybrid and two *A. porrum* varieties. Compact embryogenic callus was induced on Murashige and Skoog (MS) medium supplemented with 2,4-dichlorophenoxyacetic acid. The embryogenic calluses of all three *Allium* species were similar in appearance. For all accessions tested plants could be regenerated at a high frequency from this compact callus through somatic embryogenesis, when using kinetin supplemented MS medium (regeneration medium). Addition of abscisic acid to the regeneration medium stimulated the formation of both somatic embryos and shoots for a number of varieties. Concerning shoot regeneration from callus cultures, significant differences existed between genotypes of all accessions except one.

Abbreviations: 2,4-D – 2,4-dichlorophenoxyacetic acid, VDH – Van Der Have Seed company

Introduction

An important aim in the genetic improvement of the common bulb onion (*A. cepa* L.) is to breed for disease resistance. The closely related species *A. fistulosum* L. (Japanese bunching or Welsh onion) possesses many desirable traits (Rabinowitch & Brewster 1990) and several studies have been conducted to allow introgression of these traits into the cultivated onion. Although in the interspecific backcross (*A. fistulosum* × *A. cepa*) × *A. cepa* the transfer of genes from *A. fistulosum* to *A. cepa* has recently been reported (Cryder et al. 1991; Van der Valk et al. 1991), new bulb onion varieties containing disease resistance traits from *A.fistulosum* have not yet been produced.

Genetic manipulation techniques such as direct gene transfer and particle gun bombardment may become appropriate, alternative tools for the genetic improvement of monocotyledons including *Allium* species (Vasil 1987, 1988; Novák 1990; Potrykus 1991). However, efficient regeneration of plants from *in vitro* cultures is a major prerequisite for the successful application of these techniques. The plant regeneration efficiency from callus cultures of monocots has been shown to be dependent on various factors including type of explant, genotype, culture medium and type of callus (Vasil 1987; Novák 1990). Regenerable callus cultures of *A. cepa* and *A. fistulosum* have been obtained from various explants, including basal plates, shoot tips, onion sets, aerial bulbs, flower heads, and seed-

ling radicles (Novák et al. 1986; Novák 1990). Novák (1990) noted that B5 (Gamborg et al. 1968)-based media have been in common use for *Allium* tissue cultures. Dunstan & Short (1977) obtained a substantial increase in *A. cepa* callus growth rate after increasing the ammonium, phosphate and nitrate levels of the B5 medium. Their so-called BDS medium has served as the basal medium in a number of subsequent studies (Phillips & Luteyn 1983; Phillips & Hubstenberger 1987; Lu et al. 1989).

Somatic embryogenesis has been observed in callus cultures of *A. cepa* (Dunstan & Short 1977; Phillips & Luteyn 1983); *A. fistulosum* (Phillips & Hubstenberger 1987; Shahin & Kaneko 1986) and in F1 hybrids between *A. cepa* and *A. fistulosum* (Shahin & Kaneko 1986; Phillips & Hubstenberger 1987; Lu et al. 1989). Lu et al. (1989) modified the BDS medium to optimize shoot regeneration from basal plate callus cultures through somatic embryogenesis. *A. cepa*, *A. fistulosum* and *A. porrum* are cross-pollinated species. Therefore, mature zygotic embryos as the explant material offer the possibility to study the variation of plant regeneration capacity of callus cultures among a large number of genotypes of selected varieties. High responding individuals can then be selected for genetic transformation studies.

The aim of the present study was to establish an efficient plant regeneration system from callus cultures of *A. cepa*, *A. fistulosum*, *A. fistulosum* × *A. cepa* interspecific hybrids and *A. porrum* that could be used for future genetic transformation studies. To this end mature zygotic embryos of 17 varieties and accessions of these *Allium* species were cultured on MS medium supplemented with 2,4-D. The embryogenic potential and the regeneration capacity of the induced callus was determined on the RVP medium of Lu et al. (1989) and on Murashige & Skoog (MS) medium (Murashige & Skoog 1962), which has proved so successful for gramineous species (Vasil 1985, 1987). The effect of abscisic acid (ABA) on somatic embryo production and plant regeneration was studied because this plant growth regulator has been shown to have a promotive effect on somatic embryo development and maturation (e.g. Dunstan et al. 1988; Brown et al. 1989; Qureshi et al. 1989; Fujii et al. 1990; Roberts et al. 1990) and shoot regeneration (Rengel & Jelaska 1986; Qureshi et al. 1989; Sen et al. 1989; Sethi et al. 1990) for a range of species.

Materials and methods

Plant material

Seeds of 14 commercial varieties and three accessions comprising three *Allium* species and one interspecific hybrid were used (Table 1).

Explant material

Seeds were rinsed in 70% ethanol (30 sec) and then in tap water (30 sec) and were surface disinfested in commercial bleach (10% Na-hypochlorite), containing two drops of Tween-20 per 100 ml (1 h, continuous agitation). They were then washed in sterile tap water (ten times over a 2-h period) and stored in sterile water at 4°C for 16–40 h. They were then surface disinfested for a second time (5% Na-hypochlorite, 10 min) followed by rinsing in sterile tap water as above. Non-germinated seeds, containing firm embryos, were selected for tissue culture. A dorsal incision was made in the seed, cutting the embryo (see Fig. 1). The proximal embryo part, containing both the shoot and root apices was then extruded from the seed and cultured in a 10-cm diameter petri dish containing 25 ml of callus induction medium (Table 2). Fifteen explants were cultured per dish.

Culture media and culture conditions

The details of the tissue culture procedure are summarized in Table 2. For callus induction and growth MS medium (Sigma) was used. This was supplemented with 5.0 μM 2,4-D, 30 g l^{-1} sucrose and 3 g l^{-1} phytagel (Sigma). For plant regeneration, two media were compared:

- RVP medium according to Lu et al. (1989);
- MS medium, supplemented with 5.1 μM kinetin (MSK), with or without additional ABA.

Media were adjusted to pH 5.8 prior to autoclav-

Table 1. Origin of plant material.

Species	Variety	Origin/seed company
A. cepa	Balstora	Bejo Seeds, Noord-Scharwoude, The Netherlands
	Hyton F1[a]	Bejo Seeds, Noord-Scharwoude, The Netherlands
	Jumbo	Zaadunie, Enkhuizen, The Netherlands
	Maraton F1[a]	Van der Have, Kapelle, The Netherlands
	Norstar F1[a]	Takii & Co., Kyoto, Japan
	Oporto	Royal Sluis, Enkhuizen, The Netherlands
	Plastro	Bejo Seeds, Noord-Scharwoude, The Netherlands
	Red Baron	Bejo Seeds, Noord-Scharwoude, The Netherlands
	Sturon	Sluis & Groot, Enkhuizen, The Netherlands
	VDH 86734[b]	Valencia-type, Spain
	VDH 87906[b]	Japan
	VDH 88100[b]	partly Poland, partly France
A. fistulosum	Kincho Long White	Takii & Co., Kyoto, Japan
	Kyoto Market	Takii & Co., Kyoto, Japan
A. fistulosum × *A. cepa*	Beltsville Bunching	Stokes Seeds, Buffalo, USA
A. porrum	Porino	Nunhems Zaden, Haelen, The Netherlands
	Tilina	Zaadunie, Enkhuizen, The Netherlands

[a] F1 – hybrid
[b] accessions obtained from Van der Have Kapelle, The Netherlands.

Table 2. Procedure for callus induction and plant regeneration.

Callus culture phase	Medium[a]	Culture period (weeks)	Culture conditions
1. induction	MS1	6	25°C; dark
2. propagation	MS1	2 × 3	25°C; dark
3. regeneration	MSK ± ABA, RVP	6–8[b]	25°C; light[c]

[a] MS1, Murashige and Skoogs (MS) medium (1962), supplemented with 5.0 μM 2,4-D.
MSK, MS medium, supplemented with 5.1 μM kinetin.
RVP, medium according to Lu et al. (1989).
[b] *A. porrum*, 6 weeks; others, 8 weeks.
[c] 16 h light (ca. 25 μE m^{-2} s^{-1} cool white fluorescent light) and 8 h dark.

ing (121°C; 15 min.). ABA was dissolved in 70% ethanol and was added filter-sterilized to the autoclaved media. To allow for a quantitative determination of medium effects, embryogenic calluses (see below) from single seeds (genotypes) of all accessions were carefully divided in equal parts (5 × 5 mm) and the individual units of callus (ca. 0.12 g each) were placed on different media (2–5 units per dish, depending on the amount of callus produced per explant), and numbered. After 3 weeks of culture, calluses of 4 varieties (three *A. cepa* and one *A. fistulosum*) were examined for the presence of somatic embryos and after 6–8 weeks of culture, shoot regeneration of all accessions was recorded. Approximately 3 weeks later the majority of the plantlets could easily be transferred to soil where they were kept under high humidity for 2 weeks.

Statistical analysis

Generalized linear models (McCullagh & Nelder 1989) were used to analyze percentage of shoot-forming calluses, number of shoots per callus, and number of shoots per shoot-forming callus. Results are presented in an analysis of deviance table, and in tables that summarize means, standard errors of means and significance of differences between media effects. Some results were

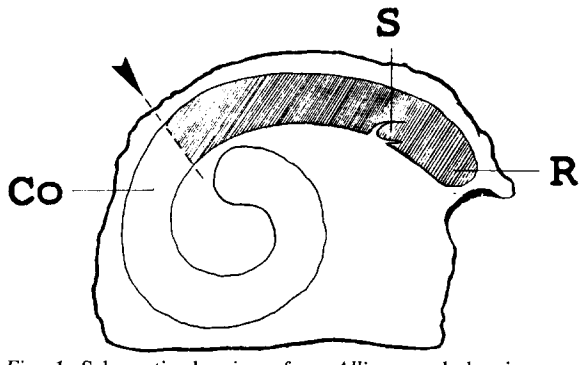


Fig. 1. Schematic drawing of an *Allium* seed showing embryo in longitudinal section (Co = cotyledon; R = root apex; S = shoot apex). The hatched area represents the explant used. The arrow points to the plane of the dorsal incision.

analyzed using a χ^2-test for equality of proportions in a 2×2 table.

Results

Callus types

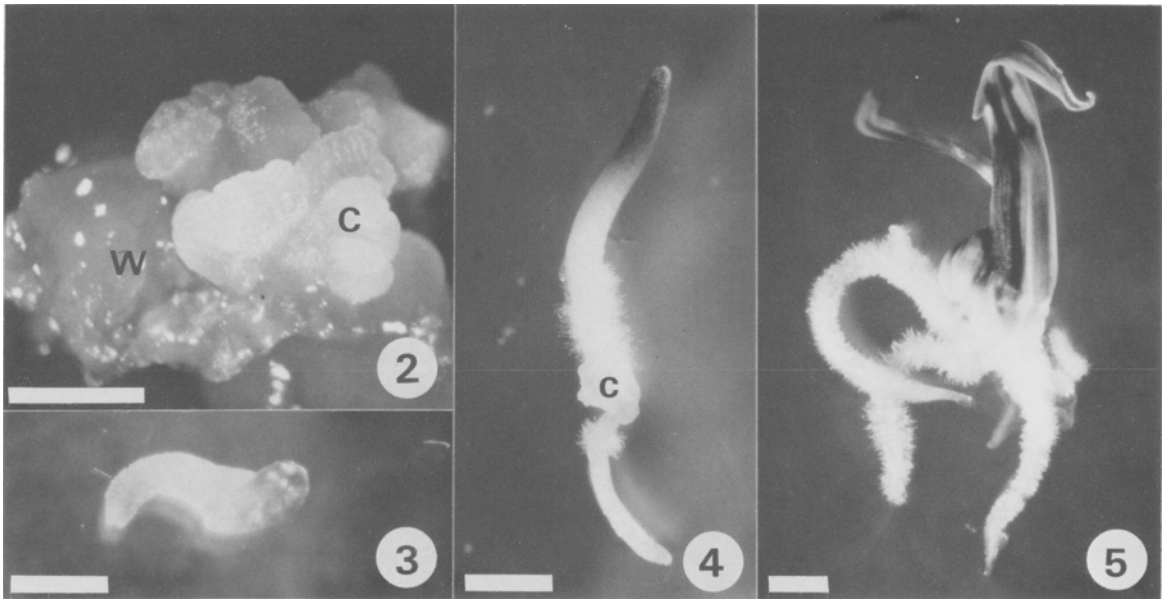
Explants of all three species produced two types of callus:
- a watery and friable type;
- a dry, compact and lobate type (Fig. 2).

The friable callus was abundantly produced by the cotyledon part of the explant whereas the compact callus was prominent at the site of the shoot apex (see Fig. 1). In a preliminary experiment the regenerative capacity of these two callus types was investigated on MSK medium using two *A. cepa* cultivars. The results presented in Table 3 show that the compact callus was significantly more regenerative than the watery callus. Therefore, compact callus was selected and cultured in all further studies.

Table 3. Shoot production by compact and watery callus on MSK medium.

Cultivar	Callus type	Number of calluses plated°	Fraction of calluses producing shoots (%)
Hyton F1	C	50	58*
	W	26	27
Oporto	C	84	67***
	W	42	31

C: compact callus; W: watery callus.
*, ***: significant at the 2 and 0.1% level, respectively (χ^2-test).
° Each callus was derived from a separate genotype. To accurately compare the tissue culture response of the two callus types, cultures initially contained the same amount of callus (0.5 gram per dish).



***Figs. 2–5.* Somatic embryogenesis in onion callus cultures (W = watery callus; C = compact callus. (Scale bars represent 2 mm).** *Fig. 2.* Two types of callus 6 weeks after initiation. *Fig. 3.* Early-stage single somatic embryo. *Fig. 4.* Two somatic embryos attached to compact callus (C). Note root hairs. *Fig. 5.* Plantlet.

Results in Table 4 shows that accessions varied strongly in the ability to produce compact callus. The VDH accessions were characterized by low induction frequencies (12–18%). The highest rates of compact callus formation (69 and 71% of the plated explants) were exhibited by the two leek varieties.

Somatic embryogenesis

Somatic embryos and embryo-like structures with the typical bipolar appearance were formed on both RVP and MSK media. Single embryos at various stages of development were prominent at the callus/medium interface after three weeks of culture (Figs 3–5). These somatic embryos germinated and developed into plantlets (Fig. 5). As early as one week after the transfer of callus to regeneration medium, early-stage embryos (Fig. 3) were present in some cultures. On the upper surface of the callus multiple embryos were frequently observed that, however, failed to germinate (not shown). On RVP medium, greening of callus without somatic embryo formation and swelling of embryos without germination were prominent in some genotypes in accessions of both *A. cepa* (Balstora, Hyton F1, Jumbo, Oporto, VDH 86734) and *A. fistulosum* (Kincho Long White).

The embryogenic capacity of compact callus was quantitatively assessed using cultures of three *A. cepa* varieties and one *A. fistulosum* variety on three media. Results in Table 5 show that the average number of somatic embryos per unit of callus per genotype was significantly higher on MSK as compared to RVP medium for all three *A. cepa* varieties. The 1.7-fold increase for the *A. fistulosum* variety was not significant. The increase in the average number of somatic embryos on MSK medium was due to both an increase in the number of responsive calluses per genotype and also to an increase in the number of somatic embryos per responsive callus (Table 5). Addition of ABA (1 μM) to the MSK medium resulted in a further increased density of somatic embryos in only one *A. cepa* variety (Hyton F1).

Shoot regeneration

Analysis of deviance (McCullagh & Nelder 1989) revealed that

- for 14 of the 17 accessions studied significant differences existed between the media with

Table 4. Production of embryogenic callus by zygotic embryos of three *Allium* species.

Species	Variety	Embryos cultured (N)	Embryos developing compact callus (%)
A. cepa	Balstora	300	21
	Hyton F1	672	54
	Jumbo	375	34
	Maraton F1	300	36
	Norstar F1	278	18
	Oporto	490	36
	Plastro	293	49
	Red Baron	266	33
	Sturon	300	38
	VDH 86734	300	12
	VDH 87906	299	18
	VDH 88100	299	15
A. fistulosum	Kincho Long White	737	28
	Kyoto Market	564	50
A. fistulosum × *A. cepa*	Beltsville Bunching	285	43
A. porrum	Porino	289	71
	Tilina	236	69

Table 5. Production of somatic embryos (SE's) by compact callus cultures of three *A. cepa* varieties and one *A. fistulosum* variety on three different media after 3 weeks of culture.

Species	Variety	Medium[1]	Genotypes cultured (N)	Calluses cultured[2] (N)	Number of SE's per callus per genotype (mean ± SEM)	Percentage of SE-producing calluses per genotype (mean ± SEM)	Number of SE's per SE-forming callus per genotype (mean ± SEM)	Percentage of SE-forming calluses that regenerated shoots
A. cepa	Hyton F1	1	40	200	0.35 ± 0.07a[3]	18 ± 3a[3]	1.70 ± 0.19a[3]	28
		2	40	200	1.08 ± 0.13b	36 ± 4b	2.58 ± 0.18b	86
		3	40	200	1.65 ± 0.16c	50 ± 4c	2.98 ± 0.17b	81
	Jumbo	1	38	148	0.59 ± 0.13a	20 ± 4a	2.88 ± 0.44a	26
		2	38	147	1.37 ± 0.20b	41 ± 4b	3.34 ± 0.34a	74
		3	38	146	1.86 ± 0.24b	51 ± 5b	3.25 ± 0.28a	83
	Oporto	1	36	162	0.39 ± 0.08a	17 ± 3a	2.07 ± 0.30a	33
		2	36	161	1.27 ± 0.16b	43 ± 4b	2.81 ± 0.25a	80
		3	36	162	1.22 ± 0.16b	38 ± 4b	2.98 ± 0.28a	63
A. fistulosum	Kyoto Market	1	40	113	2.17 ± 0.31a	50 ± 4a	3.53 ± 0.44a	42
		2	40	113	3.02 ± 0.38a	56 ± 4a	4.55 ± 0.49a	69
		3	40	106	3.09 ± 0.41a	56 ± 4a	4.42 ± 0.48a	55

[1] Media: 1, RVP; 2, MSK; 3, MSK + 10^{-6} M ABA
[2] One (unit of) callus measured 5 × 5 mm (ca. 0.12 g).
[3] Within each column treatments for each variety with different letters are significantly different at the 5% level (analysis of variance).

regard to shoot production ability (Table 6), and

- for all accessions studied, except for the leek variety Porino, significant differences existed between genotypes for shoot production from callus cultures (Table 6).

High-responding genotypes were present in all accessions studied. These were easily detected on MSK medium and were particularly abundant in Norstar F1, Sturon, VDH 88100, Kincho Long White, Beltsville Bunching, and in the two leek varieties.

Table 6. Analysis of deviance for the characters: percentage of shoot-forming calluses, number of shoots per callus, number of shoots per shoot-forming callus.

Variety	Item	df	Mean change of deviance		df	Mean change of deviance
			Percentage of shoot-forming calluses	Shoots per callus (N)		Shoots per shoot-forming callus (N)
Balstora	genotype	29	4.75*	24.48*	25	4.65
	medium	2	9.02*	47.51*	2	2.21
	residual	48	1.13	6.62	25	5.83
Hyton F1	genotype	39	2.65*	22.31*	38	8.27*
	medium	2	38.17*	199.59*	2	7.17*
	residual	78	1.18	5.43	50	2.69
Jumbo	genotype	37	2.29	19.66*	35	10.34*
	medium	2	50.56*	74.67*	2	8.61*
	residual	73	1.32	4.01	46	2.30
Maraton F1	genotype	29	5.71*	35.47*	25	7.83
	medium	2	2.26	4.76	2	0.83
	residual	48	1.11	9.08	33	6.17

Table 6 (cont.)

Variety	Item	df	Mean change of deviance		df	Mean change of deviance
			Percentage of shoot-forming calluses	Shoots per callus (N)		Shoots per shoot-forming callus (N)
Norstar F1	genotype	29	3.28*	22.49*	29	10.09*
	medium	2	28.97*	288.09*	2	61.79*
	residual	48	0.93	5.70	45	4.74
Oporto	genotype	40	5.15*	38.99*	39	13.60*
	medium	4	6.73*	53.85*	4	11.37*
	residual	140	1.17	6.36	112	4.44
Plastro	genotype	29	6.12*	40.85*	28	11.54*
	medium	4	4.88*	61.47*	4	15.35*
	residual	86	1.31	7.57	68	3.92
Red Baron	genotype	39	2.80	20.26*	38	8.74*
	medium	1	33.12*	372.40*	1	89.76*
	residual	39	1.72	5.38	26	3.37
Sturon	genotype	24	4.50*	38.86*	24	21.02*
	medium	4	12.79*	103.74*	4	23.78*
	residual	81	0.89	5.05	74	4.26
VDH 86734	genotype	17	3.93*	19.11*	15	4.02
	medium	1	2.03	9.22	1	0.15
	residual	17	1.47	6.83	9	2.48
VDH 87906	genotype	19	5.37*	21.73*	16	5.53*
	medium	1	6.12*	36.85*	1	3.46
	residual	19	1.18	3.49	11	1.42
VDH 88100	genotype	20	1.74	18.33*	20	8.55*
	medium	1	27.33*	120.37*	1	6.01*
	residual	18	1.07	7.00	16	2.72
Kincho Long White	genotype	29	3.01	42.7*	28	23.70*
	medium	1	60.66*	830.8*	1	179.93*
	residual	29	1.72	12.6	21	8.40
Kyoto Market	genotype	19	3.22*	32.80*	19	14.76*
	medium	3	3.18	36.58	3	9.69
	residual	57	1.48	8.52	57	5.42
Beltsville Bunching	genotype	29	2.91*	46.89*	29	31.78*
	medium	4	27.91*	576.89*	4	200.33*
	residual	86	1.04	11.74	82	8.59
Porino	genotype	30	4.01*	20.52	27	5.13
	medium	1	3.40	4.82	1	0.46
	residual	30	1.01	5.35	20	2.96
Tilina	genotype	29	3.38*	12.79*	27	3.63
	medium	1	6.81*	53.05*	1	16.80*
	residual	29	0.66	6.38	26	3.07

* significant at the 5% level.

Table 7. Shoot production by embryogenic callus cultures in three *Allium* species on different media.

Species	Variety	Medium[1]	Genotypes cultured (N)	Calluses[2] cultured (N)	Number of shoots per callus per genotype (mean ± SEM)	Percentage of shoot-forming calluses per genotype (mean ± SEM)	Number of shoots per shoot-forming callus per genotype (mean ± SEM)
A. cepa	Balstora	1	30	148	0.79 ± 0.17a[3]	18 ± 3a[3]	2.69 ± 0.75a[3]
		2	30	148	1.84 ± 0.27b	40 ± 5b	3.46 ± 0.77a
		3	20	98	1.69 ± 0.33b	32 ± 3b	4.02 ± 1.02a
	Hyton F1	1	40	200	0.54 ± 0.12a	14 ± 3a	3.49 ± 0.60a
		2	40	200	2.60 ± 0.27b	48 ± 3b	4.78 ± 0.38a
		3	40	200	2.85 ± 0.28b	49 ± 4b	5.17 ± 0.40a
	Jumbo	1	38	147	0.54 ± 0.13a	14 ± 3a	2.70 ± 0.52a
		2	38	147	3.02 ± 0.32b	54 ± 4b	4.76 ± 0.41b
		3	38	144	3.29 ± 0.33b	69 ± 4c	4.52 ± 0.37b
	Maraton F1	1	30	149	2.25 ± 0.32a	29 ± 3a	7.35 ± 1.54a
		2	30	149	2.59 ± 0.34a	35 ± 3a	7.02 ± 1.37a
		3	20	99	2.82 ± 0.49a	40 ± 3a	6.99 ± 1.61a
	Norstar F1	1	30	148	2.69 ± 0.47a	51 ± 6a	5.32 ± 0.80a
		2	30	148	9.26 ± 1.23b	83 ± 2b	10.53 ± 1.12b
		3	20	99	10.60 ± 1.68b	86 ± 3b	10.79 ± 1.41b
	Oporto	1	24	75	2.05 ± 0.45a	35 ± 5a	4.86 ± 0.90a
		2	41	133	4.04 ± 0.45b	58 ± 4b	6.12 ± 0.59a
		3	40	129	6.03 ± 0.56c	69 ± 4c	8.18 ± 0.68b
	Plastro	1	30	130	1.80 ± 0.32a	35 ± 5a	4.38 ± 0.63a
		2	30	130	4.00 ± 0.50bc	56 ± 6b	6.42 ± 0.66bc
		3	20	80	5.44 ± 0.81c	67 ± 7b	7.89 ± 0.95c
	Red Baron	1	40	173	1.59 ± 0.22a	37 ± 4a	3.69 ± 0.44a
		2	40	173	5.35 ± 0.41b	68 ± 4b	7.12 ± 0.47b
	Sturon	1	25	105	1.93 ± 0.36a	54 ± 3a	3.96 ± 0.66a
		2	25	105	7.67 ± 0.90b	83 ± 3b	8.73 ± 0.89b
		3	20	80	6.59 ± 0.90b	84 ± 3b	7.50 ± 0.90b
	VDH 86734	1	18	86	1.18 ± 0.44a	22 ± 8a	4.64 ± 1.64a
		2	18	86	1.65 ± 0.58a	33 ± 11a	4.74 ± 1.50a
	VDH 87906	1	20	99	0.73 ± 0.15a	18 ± 4a	2.55 ± 0.90a
		2	20	99	1.39 ± 0.24b	32 ± 8b	3.06 ± 1.03a
	VDH 88100	1	20	96	4.30 ± 1.04a	55 ± 10a	7.68 ± 1.10a
		2	20	96	9.56 ± 1.96b	85 ± 4b	10.56 ± 1.34a
A. fistulosum	Kincho	1	30	145	2.53 ± 0.60a	48 ± 7a	5.42 ± 1.02a
	Long White	2	30	145	12.16 ± 1.87b	85 ± 3b	13.39 ± 1.73b
	Kyoto	1	40	113	2.46 ± 0.51a[3]	37 ± 5a	6.12 ± 1.10a
	market	2	40	113	4.85 ± 0.75b	53 ± 5a	8.24 ± 1.22a
		3	40	106	3.76 ± 0.72ab	50 ± 6a	5.72 ± 0.88a
A. fistulosum ×	Beltsville	1	30	145	1.96 ± 0.37a	39 ± 4a	4.23 ± 0.70a
A. cepa	Bunching	2	20	145	11.07 ± 0.96b	80 ± 4b	13.37 ± 1.03b
		3	20	95	16.39 ± 1.63d	93 ± 3c	18.00 ± 1.59d
A. porrum	Porino	1	31	96	5.32 ± 0.55a	63 ± 3a	7.30 ± 0.72a
		2	31	89	5.97 ± 0.59a	75 ± 3a	7.33 ± 0.63a
	Tilina	1	30	97	6.50 ± 0.66a	77 ± 2a	8.15 ± 0.61a
		2	30	98	9.14 ± 0.78b	89 ± 1b	9.99 ± 0.62b

[1] Media: 1, RVP; 2, MSK; 3, MSK + 10^{-6} M ABA.
[2] One (unit of) callus measured 5 × 5 mm (ca. 0.12 g).
[3] Within each column treatments for each variety with different letters are significantly different at the 5% level (analysis of variance).

Table 7 shows that the average number of shoots per unit of callus per genotype after 8 weeks on regeneration medium was significantly higher on MSK medium as compared to RVP medium in 14 out of 17 accessions studied. Increases of over 3-fold were observed in Hyton F1, Jumbo, Norstar F1, Red Baron, Sturon, Kincho Long White and Beltsville Bunching. Results in Table 7 also show that the increased average shoot density on MSK is due to both an increased number of shoot-producing calluses per genotype and to an increased number of shoots per shoot-producing callus. The two *A. porrum* varieties performed well on RVP medium. Of the calluses derived from genotypes of these varieties, 63% and 77% regenerated shoots on RVP. On MSK the fraction of shoot-producing calluses increased by 12% in both *A. porrum* cultivars.

Addition of ABA at 1 μM to the MSK medium resulted in a significant increase in the average shoot density in two cultivars (Oporto, Beltsville Bunching). This increase was due mainly to an increase of the number of shoot-forming calluses (Table 7).

Inspection of the same cultures for somatic embryo production (after 3 weeks) and shoot formation (after 8 weeks) showed that, for all 4 cultivars studied, the proportion of somatic embryo-producing calluses per genotype that eventually produced shoots was 2- to 3-fold higher on MSK as compared to RVP (Table 5), indicating that maturation of somatic embryos and plant formation was clearly better on MSK as compared to RVP. This conclusion was also indicated by the significant correlation between somatic embryo formation and shoot production on MSK, with or without additional ABA, for these varieties (except Jumbo) (Table 8).

The capacity to form somatic embryos and plants decreased after prolonged subculturing of the callus. Nevertheless, for all varieties studied (Jumbo, Hyton, Oporto) green plants could be regenerated from compact callus of selected embryogenic genotypes after regular transfer for up to 15 months after culture initiation. The mode of regeneration shifted from somatic embryogenesis to organogenesis with increasing age of the callus cultures.

Plant establishment

After 10 weeks of culture on regeneration medium, well-rooted plantlets of all accessions could directly be transferred to soil after thorough rinsing in tap water. They were kept under high humidity for two weeks and could then be transferred to the greenhouse without losses.

Discussion

Somatic embryogenesis has been reported in a wide variety of monocots, including cereals such as rice, wheat, maize, barley, several grass species, and other monocots such as sugarcane, banana, lily, garlic and onion (Vasil 1985, 1987; Novák et al. 1986; Ahloowalia 1990). These studies have shown that callus that gives rise to somatic embryos is usually compact and nodular and that this embryogenic callus can be microscopically distinguished from non-embryogenic callus (Vasil 1985; Nabors et al. 1983). MS medium has been used successfully to induce embryogenic callus cultures from a large number of gramineous species (Nabors et al. 1983; Vasil 1985; Ahloowalia 1990).

Table 8. Correlation coefficients (r) between somatic embryo formation (mean number of somatic embryos per unit of callus per genotype after 3 weeks of culture) and shoot production (mean number of shoots per unit of callus per genotype after 8 weeks of culture) by embryogenic callus cultures of three *A. cepa* varieties and one *A. fistulosum* variety on three media.

Variety	Medium		
	RVP	MSK	MSK + 10^{-6} M ABA
Hyton F1	0.28*	0.86**	0.82**
Jumbo	0.54**	0.34*	0.43**
Oporto	0.44**	0.69**	0.75**
Kyoto Market	0.09	0.75**	0.38*

*, ** significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

In the present study this common MS medium proved successful for the induction of embryogenic callus cultures from three *Allium* species using zygotic embryos as the explant material. Embryogenic callus of these *Allium* species was compact and lobate as observed for gramineous species. Somatic embryogenesis and shoot regeneration was established at high frequency in these cultures using kinetin-supplemented MS medium. This medium proved superior to the complex RVP medium as recommended in a recent study for shoot regeneration from callus cultures of the interspecific hybrid *A. fistulosum* × *A. cepa* (Lu et al. 1989). Addition of ABA (1 μM) to the regeneration medium resulted in a significant increase in the number of both somatic embryos and shoots in some varieties. These observations would suggest that development and germination of somatic embryos is promoted by ABA. Stimulation of the development of somatic embryos by ABA has recently been observed in a variety of species including *Brassica*, *Datura*, *Nicotiana* (Sethi et al. 1990), *Hordeum* (Rengel & Jelaska 1986), *Picea* (Dunstan et al. 1988; Roberts et al. 1990)), *Pinus* (Sen et al. 1989), *Medicago* (Fujii et al. 1990) and *Triticum* (Brown et al. 1989; Qureshi et al. 1989). There is growing evidence that ABA directly controls the gene expression of embryogenesis proteins (e.g. Wilen et al. 1990; Hatzopoulos et al. 1990). This study has furthermore shown significant differences between genotypes of all accessions, except one, for shoot regeneration from embryogenic callus cultures.

Such strong variation between genotypes for shoot regeneration from callus cultures of selected *Allium* species has also been reported by Phillips & Hubstenberger (1987). Through the selection of highly embryogenic genotypes from a seed sample by using a simple tissue culture procedure such as that described here, excellent material for genetic transformation studies of *Allium* species may be obtained.

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