Callus Induction from Different Explants of Commercial Cultivars of Leek, *Allium ampeloprasum* var. *porrum* L.

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Abstract

Callus induction was studied for three cultivars of leek (*A. ampeloprasum* var. *porrum* L.). Compact and friable callus was induced on embryo, leaf, and root explants on 16 different media. The highest frequency of compact or friable callus formation (up to 71.4%) was obtained when mature, zygotic embryos were cultured on BDS medium, containing 30-g/L sucrose, 1.0 mg/L 2,4-D and 0.5 mg/L BA. In this paper the characterization of a friable, embryogenic callus of leek is described. This callus type was initiated on immature embryos and differed in appearance from formerly induced compact, embryogenic callus. The friable callus was comprised of numerous globular embryoids. The genotype of the donor and the different explants were important parameters in the initiation of this callus type. The basal media did not influence the friable callus production.

INTRODUCTION

There is considerable interest in plant tissue culture techniques, such as somatic hybridization, genetic transformation and in vitro selection for mutants as means to complement the leek-breeding program. For the successful application of most of these techniques the availability of an efficient procedure for plant regeneration from protoplasts or suspension cultures is a prerequisite.

In monocots, including *Allium*, it has proven difficult to culture and regenerate protoplasts that have been directly isolated from the plant (Novak, 1990; Vasil, 1983). Therefore, in our research, emphasis has been placed firstly on the initiation of callus cultures that are suitable for the establishment of morphogenic suspension cultures. To our knowledge only few data have been reported on the regeneration frequency from callus cultures of leek (Van der Valk et al., 1992).

In this report we describe callus induction in different cultivars of *Allium ampeloprasum* var. *porrum*. Different callus induction media were used and embryo, leaf and root explants from different cultivars were tested for their amenability to form morphogenic callus, in order to define the optimal conditions for callus induction and plant regeneration.

MATERIALS AND METHODS

This study was carried out during three months (from 1st October 1997 - 31st December 1997) at the Institute for Breeding of Vegetables, Medicinal and Aromatic plants, Quedlinburg, Germany.

Plant Material

Mature seeds of three *Allium ampeloprasum* var. *porrum* L. cultivars (Tropita, Blgr Herbst Gino and Blgr Winter Natan) were sterilized according to the method of Van der Valk et al. (1992).

The embryos were aseptically excised using a stereomicroscope. Leaf explants were obtained from 3-5 cm. plantlets (2 weeks old) that had previously been regenerated from mature embryo-derived callus cultures. Shoots were sliced into approximately 2 mm long sections, starting at the base of the leaf, and were referred to as explant numbers 1, 2 and 3,

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respectively. Explant 1 contained meristematic tissue. Root explants were obtained from plantlets 2 weeks old; the root explant was 2 mm long from the root tip.

Media and Culture Conditions

For callus induction the basal medium BDS (Dunstan and Short, 1977) and MS medium (Murashige and Skoog, 1962) were used. Each medium was supplemented with 30 g/L sucrose, 8 g/L agar and 8 different concentrations of 2,4-D and BA.
The concentrations were:
I1: 1.0 mg/L 2,4-D.
I2: 2.0 mg/L 2,4-D.
I3: 3.0 mg/L 2,4-D.
II1: 2.0 mg/L 2,4-D + 0.05 mg/L BA.
II2: 2.0 mg/L 2,4-D + 0.1 mg/L BA.
II3: 2.0 mg/L 2,4-D + 0.5 mg/L BA.
III1: 1.0 mg/L 2,4-D + 0.5 mg/L BA.
III3: 3.0 mg/L 2,4-D + 0.5 mg/L BA.

The media were adjusted to pH 6.0 and autoclaved. The cultures were incubated in the dark at 25°C for 6 weeks, after which the formation of compact callus was assessed. Compact calli were then transferred to fresh BDS and MS medium with the same range of concentrations of 2,4-D and BA and sub cultured at 3-weekly intervals. To compare the tissue culture response of the embryo, leaf and root explants, the explants and calli were cultured under the same conditions. To test the regeneration capacity of friable callus, 50 mg fresh weight callus was plated on MS medium, supplemented with 1 mg/L kinetin and 3-g/L phytogel and cultured at 25°C with a 16 h photoperiod (ca 3000 lux white fluorescent light).

The following data were recorded:

1. The mean number and mean percentage of different explants (embryos, leaves and roots) with callus.
2. The mean number and mean percentage of different explants (embryos, leaves and roots) with proembryogenic callus.
3. The mean fresh weight of callus per explants.
4. The density of callus produced (compact, friable and soft).
5. The characteristics of the callus produced.
6. The regeneration capacity of the callus produced.

RESULTS

Effect of Explant Type on Callus Formation

Independent of the explant type cultured, three morphologically different callus types could be distinguished: 1) a compact, cream yellow, light brown or white and nodular type with proembryogenic structures, 2) a friable type, either surrounded with water or without, cream yellow or white or light brown, 3) soft, watery and non embryogenic type.

Callus formation from embryo, leaf and root explants could be observed after 6 weeks. Data in Table 1 show that up to 100% of the embryos formed callus and up to 91% of the embryos formed compact or friable callus. Compared to callus induction on mature embryos, the frequency of callus induction on leaf or root explants was much lower. A distinct difference in the callus induction response between explants from different positions of the leaf was observed. The upper + middle explants (number 2 and 3) exhibited poor friable callus growth at low frequency (up to 53.1%). The majority of these explant showed only swelling along the cut surfaces. In contrast, up to (100 %) of the explants containing the meristem (number 1) formed callus. Compact callus formation in leaf explants (number 1) ranged from (82.1 to 90.0%) (Table 6).

Table 6 show that a high percentage (91.7-93.1%) of root explants gave callus after 6 weeks, but the mean weight of callus per root explant was very small.

Effect of Medium Composition on Callus Formation

Results in Tables l, 3 and 4 show that embryos gave a high callus response and therefore embryos were used for the comparison of different callus initiation media. The percentage of embryos that formed compact callus was lowest (22.7%) on M III 3 medium (MS + 3.0 mg/L 2,4-D + 0.5 mg/L BA) (Table 3). Maximum frequency of explants producing compact or friable callus could be achieved when embryos were cultured on BDS medium with 1.0 mg/L 2,4-D + 0.5 mg/L BA; it produced 71.4% proembryogenic callus (Table 3).

The results in Table 4 show that there was no big difference between BDS and MS basal media with respect to the percentage of proembryogenic callus formation.

Effect of Different Cultivars on Callus Induction

All three cultivars as shown in Tables 2 and 5 produced compact, friable or soft callus, although the percentage of embryos doing so varied considerably (from 49.1 % by "Blgr Winter Natan" cultivars to 82.0% embryos with callus by cultivars "Tropita"). The three cultivars showed differences in the percentage of the embryos with proembryogenic callus; the percentage was 61.3%, 34.0% and 37.5% for the three cultivars "Tropita", "Blgr Herbst Gino" and "Blgr Winter Natan", respectively (Table 2).

In general, the genotypes, within cultivars, showed differences for the amount of compact or friable callus that was formed (Tables 2 and 5). This callus type was initiated on immature embryos and differed in appearance from formerly induced compact, embryogenic callus. The friable callus was comprised of numerous globular embryoids. The genotype of the donor and the different explants were important parameters in the initiation of this callus type.

DISCUSSION

In leek, mature zygotic embryos are highly responding explants for the initiation of embryogenic callus cultures. Compared to callus induction on mature embryos, the frequency of callus induction on leaf or root explants was much lower. A distinct difference in the callus induction response between explants from different positions of the leaf was found. Two mm long leaf explants starting at the base of the leaf containing meristematic tissue gave the best results for callus induction in comparison with the upper and middle leaf explants. These exhibited poor callus growth and the majority of these explants showed only swelling along the cut surface.

The Murashige and Skoog (MS) and Dunstan and Short (BDS) media, supplemented with 1-2 mg/L 2,4-D + 0.5 mg/L BA, proved to be a suitable medium for the induction of embryogenic callus on these zygotic embryos. The embryogenic callus cultures were compact or friable, nodular and similar in appearance to those obtained for gramineous species (Vasil, 1985), other *Allium* species (Phillips and Luteyn, 1983; Van der Valk et al., 1992) and leek (Buiteveld et al., 1993).

The desired callus type was often surrounded by soft watery and non-embryogenic callus, thus making continued selection for the compact, friable callus, regeneration-competent callus type necessary. Mature and immature embryos have been successfully used to initiate embryogenic callus cultures for the major species within the Gramineae (Vasil, 1985) and within the genus *Allium* (Phillip and Luteyn, 1983; Van der Valk et al., 1992 and Buiteveld et al., 1993). The genotype of the donor and the different explants were important parameters in the initiation of this callus type.

From the test made for the regeneration capacity of friable callus, it was shown that it is possible to develop a very simple and efficient callus induction and plant regeneration system for leek.

The compact and embryogenic callus cultures derived in this study can be used to establish a cell suspension. The compact and embryogenic callus can be transferred to liquid medium, although the cultures in liquid medium kept the ability to form somatic embryos and shoots for about 6 months (Buiteveld et al., 1993).

In further experiments it should be possible to distinguish new callus types that are

morphologically different from those observed in this study. These callus types would be characterized by histological examination and the amenability for the establishment of cell suspension cultures and the subsequent isolation and culture of protoplasts would be tested.

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Tables

Table 1. Effect of different media on proembryogenic callus formation from zygotic embryos of *A.ameloprasum* var. *porrum*

cultivar	callus induction medium	No of cultured embryos	% of embryos with callus	% of embryos with proembryogenic callus	mean weight per callus (mg)
Tropita	BI1	25	100	84	35,8
	MI1	25	92	52	31,5
	BI2	22	100	91	25,8
	MI2	25	84	68	29,1
	BI3	25	68	48	19,8
	MI3	25	76	60	22,2
	BII1	25	96	32	32,1
	MII1	25	72	68	26,3
	BII2	25	76	44	45,7
	MII2	25	76	64	34,0
	BII3	48	77	52	45,9
	MII3	50	76	60	39,3
	BIII1	25	92	80	65,5
	MIII1	25	80	60	48,5
	BIII3	25	88	80	46,3
	MIII3	25	72	44	57,6
Blgr Herbst Gino	BI1	25	80	40	32,2
	MI1	25	60	40	44,1
	BI2	25	80	12	28,1
	MI2	24	58	12	37,8
	BI3	25	44	28	23,4
	MI3	25	36	12	19,0
	BII1	25	84	36	38,5
	MII1	25	92	28	37,8
	BII2	25	84	40	39,0
	MII2	25	60	44	33,8
	BII3	50	62	52	39,8
	MII3	50	54	28	35,7
	BIII1	20	70	70	25,0
	MIII1	25	68	52	28,1
	BIII3	25	44	32	21,6
	MIII3	25	20	12	41,4
Blgr Winter Natan	BI1	25	40	28	25,8
	MI1	25	32	24	33,5
	BI2	25	68	28	21,4
	MI2	25	44	24	15,0
	BI3	25	12	12	14,7
	MI3	25	60	32	20,1
	BII1	25	16	16	22,7
	MII1	25	80	72	43,0
	BII2	25	20	8	19,8
	MII2	25	96	72	36,7
	BII3	50	82	64	31,0
	MII3	50	42	40	39,7
	BIII1	25	68	64	25,2
	MIII1	25	32	24	43,0
	BIII3	25	52	52	30,5
	MIII3	25	16	12	40,7

proembryogenic callus - compact, friable, friable/compact

Table 2. Effect of the genotype of donor plant on proembryogenic callus formation of zygotic embryos, irrespective of the callus induction media used.

Cultivar	*N*o of cultured embryos	Embryos with callus		Embryos with proembryogenic callus		Mean weight per callus (mg)
		no	%	no	%	
Tropita	445	365	82.0	273	61.3	38.4
Blgr Herbst Gino	444	274	61.7	151	34.0	33.4
Blgr Winter Natan	450	221	49.1	169	37.5	29.6

Table 3. Effect of different media on callus formation from zygotic embryos of *A. ameloprasum* var. *porrum*, irrespective of the genotype used.

Callus induction medium	*N*o of cultured embryos	*N*o of embryos with callus	% embryos with callus	*N*o of proembryogenic callus	% of proembryogenic callus	Mean weight per callus (mg)
BIl	75	55	73.3	38	50.7	31.3
MI1	75	46	61.3	30	40.0	36.4
BI2	72	59	81.9	30	41.7	25.1
MI2	74	46	62.2	26	35.1	27.3
BI3	75	31	41.3	22	29.3	19.3
MI3	75	43	57.3	26	34.7	20.4
BII1	75	49	65.3	21	28.0	31.1
MII1	75	61	81.3	42	56.0	35.7
BII2	75	45	60.0	23	30.7	34.8
MII2	75	58	77.3	46	61.3	34.8
BII3	148	109	73.6	83	56.1	38.9
MII3	150	86	57.3	64	42.7	38.2
BIII1	70	54	77.1	50	71.4	38.6
MIII1	75	45	60.0	34	45.3	39.8
BIII3	75	46	61.3	41	54.7	32.8
MIII3	75	27	36.0	17	22.7	46.6

Table 4. Effect of basal medium on proembryogenic callus initiation from mature embryos of leek irrespective of the genotype used

Basal medium	*N*^{o} of cultured embryos	*N*^{o} of embryos with callus	% embryos with callus	*N*^{o} of proembryogenic callus	% of proembryogenic callus	Mean weight per callus (mg)
MS	674	412	61.1	285	42.3	35.3
BDS	665	448	67.4	308	46.3	32.3

Table 5. Proembryogenic callus induction from mature embryos of various cultivars of leek on the different basal media

cultivar	basal medium	*N*° of cultured embryos	*N*° of embryos with callus	% embryos with callus	*N*° of proembryogenis callus	% of proembryogenic callus	mean weight per callus (mg)
Tropita	MS	225	176	78.2	136	60.4	36.4
	BDS	220	189	85.9	137	62.3	40.3
Blgr Herbst Gino	MS	224	125	55.8	64	28.6	34.8
	BDS	220	149	67.7	87	39.5	31.9
Blgr Winter Natan	MS	225	111	49.3	85	37.8	34.6
	BDS	225	110	48.9	84	37.3	24.7

Table 6. Callus initiation from different explants of A. *anapeloprasum* var. *porrum* L. on MS medium containing l mg/L 2.4-D.

Explant	Cultivar	Explants cultured (No.)	Explants with callus (No.)	(%)	Explants with proembryogenic callus (No.)	(%)	Mean weight of callus/leaf-explant (mg)
Leaf 1	Blgr	78	74	94.9	64	82.1	79.1
2	Herbst	27	20	74.1	9	33.3	v.s.
3	Gino	28	15	53.6	3	10.7	v.s.
Root		29	27	93.1	4	13.8	v.s.
Leaf 1	Blgr	30	30	100.0	27	90.0	82.4
2	Winter	32	23	71.9	17	53.1	v.s.
3	Natan	37	8	21.6	5	13.5	v.s.
Root		60	55	91.7	47	78.3	v.s.

v.s.: very small