CARIBBEAN FOOD CROPS SOCIETY

39

Thirty Ninth
Annual Meeting 2003

Grenada

Vol. XXXIX Number 2

Proceedings of the Caribbean Food Crops Society. 39(2):267-271. 2003

CALLUS INDUCTION AND SHOOT PRODUCTION IN *CAPSICUM CHINENSE* (POD TYPE SCOTCH BONNET)

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ABSTRACT: Various explants excised from greenhouse-grown and in vitro-germinated seedlings of *Capsicum chinense* were inoculated onto MS medium supplemented with different concentrations of 2,4- dichlorophenoxyacetic acid (2,4-D) and benzyladenine (BA) or BA and indolacetic acid (IAA) treatments, to allow callus induction and shoot formation, respectively. All cultures were incubated at 28° C with a 16-hour photoperiod (3000 lux). All 2,4-D / BA treatments induced callus on stem, hypocotyls, and leaf explants. The 0.8 mg/L 2,4-D and 1.5 mg/L BA, 2.0 mg/L 2,4-D and 3.0 mg/L BA and 3.2 mg/l 2,4-D and 1.5 mg/L BA treatments gave the best results. The calli produced were of compact, friable or mixed types. Shoot tip and nodal explants produced shoots on all BA/IAA treatments and the control; bud burst ranged from 40% to 100%. IAA 1 mg/L and BA 8 mg/L gave 100% budburst and IAA 1 mg/L and BA 1mg/l gave the greatest shoot height. No shoots were produced directly on leaf, hypocotyls, or seed leaf explants. The response of explants derived from greenhouse and that of those of in vitro origin were compared.

INTRODUCTION

There have been extensive studies conducted on callus induction and shoot production from explants of *Capsicum annuum*. The purpose of such study has an important role to play in establishing a regeneration protocol by determining the specific optimal conditions for plant regeneration.

This present investigation of *Capsicum chinense,* a member of the *Capsicum annuum* species complex, is modeled in part after successful attempts at callus induction of *Capsicum annuum* L. using leaf tissue (Kintios et al., 1996) and shoot production using nodal and shoot tip explants (Christopher et al., 1994).

The aim of this investigation is to determine the specific effects of manipulating explant source, explant type, and growth regulator concentration on callus induction and shoot production of *Capsicum chinense,* and to compare these effects as a means toward future development of a regeneration protocol.

MATERIALS AND METHODS

The *Capsicum chinense* pod type Scotch Bonnet was used in the study. Donor plants were obtained from two sources. The first source was greenhouse grown seedlings treated with Torque™, Commando™, and Neemco™ in the first, second, and third weeks, respectively, prior to use. The second source of plants was seedlings germinated from seeds treated with 20% sodium hypochlorite supplemented with two drops of Tween 20 for 20 minutes.

The various media were prepared by using the standard Murashige and Skoog basal medium supplemented with growth regulators, 3% sucrose, and 0.8% agar, adjusted to pH 5.8 and autoclaved at 121° C for 20 minutes. The callus induction media were supplemented with 0.8 mg/L 2,4-D and 1.5 mg/L BA; 0.8 mg/L 2,4-D and 3.0 mg/L BA; 0.8 mg/L 2,4-D and 4.5 mg/L BA; 2.0 mg/L 2,4-D and 3.0 mg/L BA; 2.0 mg/L 2,4-D and 4.5 mg/L BA; 3.2 mg/L 2,4-D and 1.5 mg/L BA); 0.0 mg/L 2,4-D and 0.0 mg/L BA, designated MS callus induction media 1, 2, 3, 4, 5, 6, and 7.

The shoot induction media were supplemented with 1.0 mg/L IAA and 1.0 mg/L BA; 1.0 mg/L IAA and 2.0 mg/L BA; 1.0 mg/L IAA and 4.0 mg/L BA; 1.0 mg/L IAA and 8.0 mg/L BA; 1.0 mg/L IAA and 12.0 mg/L BA; 0.0 mg/L IAA and 4.0 mg/L BA; 0.0 mg/L IAA and 0.0 mg/L BA; designated MS shoot production media 1, 2, 3, 4, 5, 6, and 7.

Leaf discs, stem, and shoot tip explants were excised from the greenhouse seedlings and sterilized with 10% sodium hypochlorite supplemented with Tween 20 for 10 minutes and then rinsed in three changes of sterilized distilled water prior to use. Leaf, hypocoltyl, cotyledon, nodal, and shoot tips were also excised from the in vitro germinated seedlings

The greenhouse derived leaf and stem tissues as well as in-vitro derived leaf and hypocotyls tissues were placed on the callus induction media at ten replicates per medium. Greenhouse derived nodes and shoot tips as well as in vitro-derived leaf, cotyledon, hypocotyls and nodes, and shoot tips were placed on the shoot production media at ten replicates per medium. The explants were then placed under conditions of 26.56 V/m^2 light intensity and 16-hour photoperiods for four weeks.

Results were analyzed in terms of percentage contamination, percentage callus induction and callus texture, and percentage bud burst and shoot height.

RESULTS

Greenhouse derived explants that were subjected to the sterilization procedure succumbed to both bacterial and fungal contamination at 1.5%, 1.4%, and 11.4% for leaf discs, stem, and nodal and shoot tip explants respectively. Also, 14.7% of the leaf tip explants were further lost because of the high concentration of a sodium hypochlorite. There were no further such losses among the other explant types.

Calli were induced on each of the greenhouse derived explants in all the media except the control after four weeks. Table 1 indicates the results of the callus induction treatments for greenhouse derived leaf explants.

Table 1. Greenhouse leaf discs and the percentages and types of calli induced in the seven media after four weeks.

Medium			Callus type %		
2,4-D:BA mg/L (approx. ratio)			Compact	Mixed	Friable
1	0.8:1.5	(0.5)	55.5	33.3	11.1
2	0.8:3.0	(0.3)	60.0	40.0	0
3	0.8:4.5	(0.2)	80.0	20.0	0
4	2.0:3.0	(0.7)	50.0	37.5	12.5
5	2.0:4.5	(0.4)	40.0	60.0	0
6	3.2:1.5	(2.0)	22.2	33.3	44.4
7	0:0		0	0	0

Most of the calli induced on the leaf discs were compact. The greatest percentage friable calli was achieved on Medium 6 (3.2 mg/L 2,4-D and 1.5 mg/L BA). Medium 1 induced calli first and achieved the largest calli within the duration of the experiment.

Table 2 shows that a greater percentage of friable calli were induced on stem tissue at 90% for Medium 1 (0.8 mg/L –1 2, 4-D and 1.5 mg/L BA) and Medium 4 (2.0 mg/L 2,4-D and 3.0 mg/L BA); and at 100% for Medium 6 (3.2 mg/L 2,4-D and 1.5 mg/L BA).

Table 2. Showing Greenhouse stems and the percentages and types of calli induced in the seven media after four weeks.

Medium			Callus type %		
2,4-D:BA mg/L (approx. ratio)			Compact	Mixed	Friable
1	0.8:1.5	(0.5)	0	10	90
2	0.8:3.0	(0.3)	100	0	0
3	0.8:4.5	(0.2)	90	10	0
4	2.0:3.0	(0.7)	0	10	90
5	2.0:4.5	(0.4)	0	40	60
6	3.2:1.5	(2.0)	0	0	100
7	0:0		0	0	0

Medium 1 again induced calli first but Medium 6 produced the largest calli within the duration of the experiment. Also, 100% friable calli were induced on each of the in vitro-derived explants in all the media except the control.

Like the greenhouse derived explants, Medium 1 induced calli first whereas Medium 6 produced the largest calli for both explant types within the duration of the experiment. Tables 3 and 4 show results for callus induction using in vitro-derived explants.

Table 3. In vitro leaves and the percentages and types of calli induced in the seven media after four weeks.

Medium			Callus type %		
2,4-D:BA mg/L (approx. ratio)			Compact	Mixed	Friable
1	0.8:1.5	(0.5)	0	0	100
4	2.0:3.0	(0.7)	0	0	100
6	3.2:1.5	(2.0)	0	0	100
7	0:0		0	0	0

Table 4. In vitro hypocotyls and the percentages and types of calli induced in the seven media after four weeks.

Medium			Callus type %		
2,4-D:BA mg/L (approx. ratio)			Compact	Mixed	Friable
1	0.8:1.5	(0.5)	0	0	100
4	2.0:3.0	(0.7)	0	0	100
6	3.2:1.5	(2.0)	0	0	100
7	0:0		0	0	0

From the results of the experiment it was evident that the greatest shoot height was achieved from nodal and shoot tip explants derived from in vitro-grown seedlings at 10.9 ±1.99 mm in Medium 2 (1 mg/L IAA and 2 mg/L BA). This was significantly greater than the 4.0 ± 1.00 mm-shoot height achieved in Medium 1 (1mg/L IAA and 1 mg/L BA). These results are indicated in Table 5 below.

Table 5. Percentages bud burst and mean shoot height/mm after 22 days for greenhouse and in vitro-grown nodes and shoot tips for the seven media.

Medium		In vitro nodes & shoot tips		Greenhouse nodes & shoot tips	
	growth regulators [iaa]/[ba]mgl-1	percentage bud burst	mean shoot height/mm and standard error	percentage bud burst	mean shoot height/mm and standard error
1	1:1	80	4.0 ± 1.00	100	6.4 ± 1.41
2	1:2	80	3.4 ± 0.77*	100	10.9 ±1.99
3	1:4	80	2.3 ± 0.92	90	4.3 ±1.02
4	1:8	90	2.8 ± 0.94*	70	1.0 ± 0.63
5	1:12	50	0.7 ± 0.66	100	6.6 ± 2.17*
6	0:4	50	3.7 ± 2.22	100	2.5 ± 0.65
7	0:0	40	0.6 ± 0.38	80	3.0 ± 1.05

*explant(s) exhibited proliferation.

Two media of the shoot production treatment containing greenhouse-derived explants exhibited proliferation in Medium 4 (1mg/L IAA and 8 mg/L BA) in four explants and in Medium 2 (1mg/L IAA and 2 mg/L BA) in one explant. Medium 5 (1mg/L IAA and 12 mg/L BA) of the shoot production treatment containing in vitro-derived explants also showed proliferation.

DISCUSSION

The sterilization protocol proved effective for the stem tissue since the contamination percentages were low. However, even though the contamination percentages were also low for the leaf tissue, the concentration of, and exposure to, the sodium hypochlorite seemed to have been too damaging to the leaf tissue. There was a significant loss of tissue to the sterilization procedure because leaf tissue is much thinner and more delicate than the stem tissue. Conversely a significant percentage of the nodal and shoot tip explants succumbed to contamination because the crevices within the nodes and tips harboured fungi and bacteria which could not be penetrated by the sodium hypochlorite solution.

Calli were induced in each of the callus-inducing media except the control, which contained no growth regulators, all of which suggests that growth regulators are required for callus induction. From the results of the callus induction experiment it appears that the degree of callusing is proportional to the ratio 2,4-D to BA concentration. 2,4-D is an auxin, which, in addition to its role as a root inducer, also induces callus development. For the greenhouse-derived explants, Medium 6 (3.2 mg/L 2,4-D and 1.5 mg/L BA) produced the greatest percentage friable calli in every case and contained the highest concentration 2,4-D as well as the highest 2,4-D: BA ratio at 2.0. Friable calli are useful in tissue culture since the callus is easily divided,

for transfer to other medium or any similar manipulation. Compact calli are less readily divided and mixed calli share similar properties with both friable and compact calli. It appears that the higher the 2,4-D ratio the greater the percentage of friable calli.

In addition, in each case calli were induced first in Medium 1 (0.8 mg/L 2,4-D and 1.5 mg/L BA) which contained the lowest concentration of either growth regulator, all of which suggests that low exogenous growth regulator concentrations, but not a complete absence of growth regulators, is required to initiate callus induction.

Also 100% friable calli were induced with either in vitro explant type since the younger tissue responded more effectively to the exogenous growth regulators than the greenhouse-derived explants in forming calli.

The results of the shoot production treatment showed that there was a greater overall percentage of bud burst achieved with the nodal and shoot tip explants. Higher percentages of bud burst were achieved at lower concentrations of BA for both explant types except the in vitro explants in Medium 5 (1 mg/L IAA and 12 mg/L BA). Both explant types seem to have responded well to growth regulator concentrations at the lower end of the BA range. It seems that the higher concentrations of BA inhibited bud burst, as well as the increase in shoot height for both explant sources. The explants responded to the control media, all of which suggests that they are producing endogenous growth regulators and require only a minimal additional amount of BA to achieve bud burst or an increase in shoot height once bud burst has taken place.

The greatest shoot height was achieved using in vitro-derived nodes and shoot tips in medium containing 1mg/L IAA and 1 mg/L BA. This represents a lower BA concentration than that in which the greatest shoot height was achieved for the greenhouse-derived explants. Two media of the shoot production treatment containing greenhouse derived explants exhibited proliferation in Medium 4 (1mg/L IAA and 8 mg/L BA) in four explants; in Medium 2 (1mg/L IAA and 2 mg/L BA) in one explant. Medium 5 (1mg/L IAA and 12 mg/L BA) of the shoot production treatment containing in vitro-derived explants also showed proliferation in two explants. Proliferation seems to take place at higher BA concentrations for each explant source.

Again in vitro-derived explants seemed to be more tolerant of higher BA concentrations than greenhouse-derived explants. Proliferation of shoots is useful in tissue culture since multiple shoots in this case are produced from one explant. This is especially useful if the explant contains the desired traits.

Only one of the hypocotyl explants produced a shoot since this explant type does not contain pre-formed meristems. Pre-formed meristems are groups of localized actively dividing cells that give rise to root and shoot systems. A high percentage of shoots was not expected from this explant type.

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