OnLine Journal of Biological Sciences 1 (8) : 720-722, 2001

Shoot Regeneration from Wasabi (*Wasbia japonica* Mtsum) Callus

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Abstract: Adventitious shoot formation was induced on callus of wasabi. Adventitious buds were formed from subculture of callus on modified MS media (containing 0.002 mg/ l TDZ (n-phenyl-N- 1, 2, 3- thidiazol-5-yl-urea or thidiazuron) and 1 mg/ l of NAA (naphhaleneacetic acid) were failed to elongate. Bud forming calli were subcultured in modified MS media containing BA, (6-benzyladenine) or kinetin with or without NAA or NAA alone. Shoot development was observed on the enlarged callus in media containing BA or kinetin with NAA. While media containing BA or kinetin without NAA and media containing only NAA did not result in organ formation. Calli subcultured on hormone free media caused the buds to become black and die out. Shoots formed in the presence of kinetin were either smaller in size or turned brown in colour. Green and plump shoots were formed in media containing 0.5 or 1 mg/ l of BA with 0.5 mg/ l of NAA. Higher doses of BA and NAA caused the shoots to become thin and branched. Calli that produced the best shoots also grew faster than others having a lush green appearance. However, root formation was less frequent in shoot forming calli. Root formation was most frequent when kinetin was used as a source of cytokinin.

Key words: Wasabi, cruciferae, cytokinin, TDZ, shoot regeneration

Introduction

Wasabi (member of Cruciferae family) is a traditional Japanese condiment and is mostly grown in Japan. Stringent environmental requirements and rather slow growth habit limit the production of wasabi. Wasabi propagation is mainly accomplished with offshoots. But a few offshoots grow large enough for transplanting from one mother plant within two years of field growth. Diseases are often passed in the new crop from the preceding crop, reducing the vitality of the plants. Seedlings are usually effective to break the disease cycle. But raising seedling is rather cumbersome and requires longer time. Cross pollination results in losing the desired characteristics of the mother plant. Thus, seedlings make up an ununiform crop. Micro propagation has been suggested but bacterial contamination is rather high in case of direct explanting (Matsumoto *et al.*, 1995). Plant regeneration of wasabi from callus culture has not been reported yet.

Since the discovery of the cytokinin - like activity of thidiazuron (TDZ), it has been used for Micro propagation of a wide range of woody species because of its tremendous ability to stimulate shoot proliferation (Huetteman and Preece, 1993). However, TDZ use in cruciferous plants are only limited to *Arabidopsis thaliana* (Gleddie, 1989), cabbage (Souza *et al.*,1998) and broccoli (Mok, *et al.*, 1987). In this paper the first time shoot regeneration of wasabi from callus subculture through the use of TDZ, was investigated.

Materials and Methods

Wasabi callus was initiated from stem explants cultured on Murashige and Skoog (MS) media supplemented with 3% sucrose and solidified with 0.2% Gelrite. The media contained 1 mg l^{-1} of both BA (6-benzyladenine) and NAA (∝-naphthalene acetic acid). Subsequently callus was proliferated on the same media. Explants from these proliferated calli were cultured in test tubes containing 10 ml of MS medium (half strength of basic macro and micro nutrient salts and full strength of iron and vitamins). The media was supplemented with 3% sucrose and solidified with 0.2% Gelrite and contained 0.002 mg/ l of TDZ and 1 mg/ l of NAA. The cultures were incubated at 15°C under 14 h of photoperiod with a light intensity of 30 μ mol m^{-2} $s^{-1}$for 8 weeks. Numerous buds were formed on the calus explants. This morphogenic calli was then excised into (3 mm X 3 mm X 3 mm) segments and subcultured in culture bottles containing 100 ml of modified MS medium. The media contained BA or kinetin (either at 0, 0.5, 1 or 2 mg/ l) with NAA (either at 0, 0.5 or 1 mg/ l). The culture was incubated at 5°C and semi dark condition for six weeks and then in 15°C under 14 hrs of photoperiod with a light intensity of 30 μ mol m^{-2} s^{-1} provided by cool white fluorescent light. 30 explants were used for each treatment culturing 6 explants / culture bottle. Cultures were evaluated 20 weeks later.

Results and Discussion

Subcultured morphogenic calli responded very slowly. Explants cultured in medium containing either BA or kinetin along with NAA produced modified shoots without leaves or roots (Fig. 1). Shoots were also produced in media containing 2 mg/l of kinetin without NAA, but they had a black appearance implying dead. Media containing cytokinin or auxin alone failed to produce any shoots (Table 1). But shoots produced from the use of BA were rather more plump and lush green than those were produced from the use of kinetin (Fig. 1). When kinetin was used in the medium, the shoots were thin, often brown in colour, and too branched. Kinetin produced green shoots only when applied at 1 mg/l concentration in combination with the same amount of NAA. In the case of BA application, callus growth was also profuse in the treatments producing plump shoots (BA at 0.5, 1 and 2 mg/ l with 0.5 mg l^{-1} NAA). In these cases calli grew into lush green compact masses. The diameters of the calli masses measured after 20 weeks of culture were also greatest among all other treatments tested (Table 1). BA with 0.5 mg l^{-1} each and NAA resulted in the highest percentage of calli producing shoots (35%). However, more plump shoots were produced with 1 mg l^{-1} of BA and 0.5 mg/ l of NAA, although, the percentage of shoot producing calli was lower (20%). Also, the number of shoots /explant was higher in the latter case (0.53 /explant) than the farmer. In the similar treatments when kinetin was used instead of BA, the percentage of calli producing shoots and number of shoots/ explant were very low (Table 2). Root formation was low when shoots were produced under the influence of BA compared to that under the influence of kinetin. Media without any hormone treatment caused the subcultured calli to turn partially brown, with buds becoming

Table 1: Morphogenesis in callus subculture in regulator free medium and medium containing BA with or without NAA after 20 weeks.

Regulators		Callus diameter (mm)[x]	Shoot formation *	No.shoot / explant[x]	Bud formation *	No. of Bud./ explant [x]	Root formation[y]	No. of root./explant[x]		
BA) (mg/l)	NAA (mg/l)							Normal	abnormal	Total
0	0	5.83±0.17	0	0	2.78	0.06±0.06	50.00	0	0	3.81±0.88
0	0.5	8.22±0.16	0	0	65.57	5.29±0.88	85.25	2.6	0.8	5.37±0.590
	1	7.91±0.23	0	0	70.49	5.25±0.62	49.18	1.5	1.1	2.62±0.43
0.5	0	7.06±0.34	0	0	0	0	0	0	0	0
1	0	5.79±0.17	0	0	0	0	0	0	0	0
2	0	6.12±0.22	0	0	0	0	0	0	0	0
0.5	0.5	11.20±0.53	34.78	0.48±0.15	82.61	3.65±0.72	30.43	0.1	0.5	0.61±0.23
1	0.5	11.08±0.57	20.00	0.53±0.26	73.33	2.37±0.36	0	0	0.2	0
2	0.5	10.67±0.55	17.24	0.28±0.13	75.86	2.62±0.55	0	0	0.1	0
0.5	1	7.96±0.15	3.33	0.03±0.03	53.33	1.43±0.45	10.00	0	0.2	0.17±0.10
1	1	6.66±0.23	0	0	80.65	5.74±0.84	0	0	0	0
2	1	7.04±0.20	16.7	0.29±0.11	75.00	6.08±1.09	0	0	0.04	0.04±0.04

[x]mean value ± SE [y]percentage of the explant that could produce shoot/bud/root

Table 2: Morphogenesis in callus subculture in medium containing kinetin with or without NAA after 20 weeks.

Regulators		Callus diameter (mm)[x]	Shoot formation*	No.shoot explant[x]	Bud formation[y]	No. of Bud./ explant [x]	Root formation[y]	No. of root./explant[x]		
BA) (mg/l)	NAA (mg/l)							Normal	abnormal	Total
0.5	0	6.52±0.23	0	0	22.58	0.81±0.31	77.42	2.2	2.4	4.58±0.77
1	0	5.53±0.18	0	0	19.35	0.55±0.24	80.65	0.9	1.5	2.35±0.38
2	0	6.18±0.20	6.67	0.07±0.05	13.33	0.43±0.24	76.67	1.0	2.0	2.97±0.70
0.5	0.5	7.97±0.25	3.33	0.07±0.03	26.67	0.57±0.20	100.00	1.4	3.8	5.20±0.64
1	0.5	9.16±0.31	6.89	0.07±0.05	17.24	0.17±0.07	93.10	2.8	2.0	4.83±0.68
2	0.5	8.60±0.34	3.33	0.03±0.03	26.67	0.80±0.27	83.33	1.0	2.4	3.37±0.52
0.5	1	8.27±0.39	0	0	35.48	1.87±0.54	82.14	2.3	2.5	4.77±0.87
1	1	7.91±0.18	25.00	0.46±0.17	50.00	1.32±0.31	75.00	0.5	2.0	2.46±0.46
2	1	8.67±0.34	0	0	70.83	2.54±0.45	75.00	0.8	2.0	2.79±0.66

[x]mean value ± SE [y]percentage of the explant that could produce shoot/bud/root

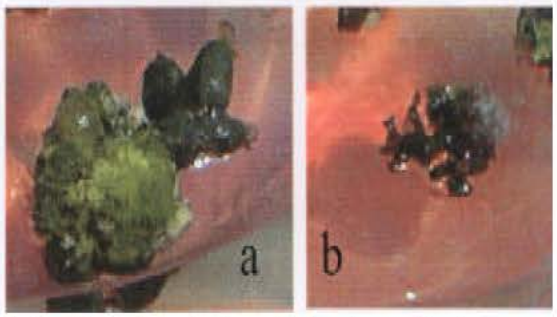

Fig.1: Shoot formation in subcultured wasabi callus.
a: Under the influence of 1mg/lBA and 0.5 mg l^{-1} NAA.
b: Under 1mg l^{-1} of both kinetin and NAA

black in colour, appearing to die. BA without NAA also caused the subcultured calli to turn fully brown or black or at least partly causing the buds to die. There were no further bud or root developments in these treatments. While under the influence of Kinetin alone, subcultured calli also developed similar appearances in colour; but with 13 to 20% calli producing new buds. Root formation was enhanced in these cases (about 80% of the explants producing roots, with 2.4 to 3 roots /explant). In hormone free medium and in medium with cytokinin alone, callus growth was the least evident. When NAA alone was used in the medium without any cytokinin, callus appeared to be green and sometimes partly brownish. Callus growth was better than under the influence of cytokinin alone, with new bud and root production in abundance. Buds were formed in 65-70% cases with about 5 buds/explant and roots in 49-85% cases with 2.6-5.4 roots/explant. NAA application at 1 mg/ l concentration with BA at 0.5 mg/ l produced only one brownish and frail shoot; and with 1 mg/ l BA concentration failed to produce any shoots. In both cases, buds turned pale green or brownish in color along with a similar appearance in the calli. Bud production was abundant (53-80% of explant) with a maximum of 5.7 buds per explant, but root production was almost absent. BA at 2 mg/ l with 1 mg/ l of NAA produced some shoots (16.7% cases) but they were thin and black in appearance. Bud production was huge (75% cases, 6 buds /explant), without root formation. In the case of kinetin (0.5, 1 or 2 mg/ l) along with 1 mg/ l of NAA, the callus mostly remained green and produced a good deal of buds (35.5-70% of explants) with a maximum of 2.5 buds/explant. In these cases root production was fairly high. Kinetin and NAA both at 1 mg l^{-1} concentration gave the highest number of shoots/explant (and also the highest percentage of shoot producing calli) among all kinetin treatments, but shoots were mostly brownish and small.

In preliminary experiments, buds produced from TDZ application on calli and direct explant culture failed to elongate. Fellman *et al.* (1987) also reported that bud proliferation at 10^{-6} M thidiazuron in petunia leaf segment culture was profuse but shoot elongation was suppressed, assuming that the strong cytokinin activity was the probable cause. Bud forming calli subcultured on growth regulator free medium, which, is supposed to wear off the TDZ activity, also failed to cause the buds to elongate, and rather degenerated them. In contrast, a second cytokinin was found to be important for the survival and further development of the buds formed under the influence of TDZ. Nielsen *et al.* (1993) reported a synergistic effect of TDZ and BA in *Miscanthus sinensis* 'Gigantis axillary shoot formation through sequential

application. Successful application of the use of a primary medium containing TDZ for adventitious shoot induction followed by a secondary medium with other plant growth regulators to promote shoot development in *Rhododendron* has been reported by Preece and Imel (1991). In addition NAA as a auxin source was found indispensable for shoot development. Jain *et al.* (1988) also observed for several *Brassica* species (*B. juncea, B. campestris and B. carinata*) that plant regeneration frequently declined sharply in the absence of auxins in the medium. Mok *et al.*, 1987, reported abnormal growth and vitrification in broccoli when TDZ was applied at a concentration of 10μM. An improper dose of TDZ might be responsible for the abnormal shape of the shoots developed in experiment. This experiment was the first in regenerating shoots from wasabi callus subculture. Now further trials to refine the method in order to promote normal shoot development, were carried out.

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