Kasetsart J. (Nat. Sci.) 40 : 625 - 633 (2006)

Production of Salt Tolerance Dwarf Napier Grass (*Pennisetum purpureum* cv. Mott) Using Tissue Culture and Gamma Irradiation

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ABSTRACT

As high as 95% of callus was induced when young leaves of dwarf Napier grass were cultured in MS medium supplemented with 5% coconut water and 2 mg/l 2,4-D. These calli were of highly compact type. Upon transferring these calli to grow on the plantlet induction medium, it was found that the MS medium containing 5% coconut water, 1 mg/l NAA, 0.5 mg/l BAP and 0.5 mg/l 2,4-D gave the best average plantlet production of 58.8%. Irradiated calli at the levels of 0, 10, 20, 30, 40 and 50 Gy were subject to culturing growth on the selected plantlet induction medium containing different NaCl concentrations of 0, 0.5, 1.0, 1.5 and 2.0%. The total of 108 clones could survive in the salt supplemented medium. These surviving clones were then transferred to grow in the field at National Corn and Sorghum Research Center, Nakorn Rachasima province. Twenty-one of good characteristic clones were selected from the field to be further grown at high salinity soil in Borabue district, Maha Sarakarm province and 8 clones were finally chosen as salt tolerance. DNA fingerprinting patterns of the 21 selected clones were analyzed using AFLP technique with 11 pairs of primers. The patterns were significantly different at high percentage of polymorphism (94.75%). The similarity index among these samples ranged from 0.569 to 0.784 indicating that the morphological difference among them was the result of true mutation induced by irradiation and tissue culturing.

Key words: dwarf Napier grass, salt tolerance, tissue culture, gamma irradiation, DNA fingerprint, AFLP

INTRODUCTION

Dwarf Napier grass, *Pennisetum purpureum* cv. Mott is considered a cross-pollinated plant. It was originally found in Florida, USA. This plant is an allotetraploid with chromosome number of 2n=4x=28. Dwarf Napier grass contains several positive characters which are beneficial for animal feeding, i.e., they can be grown for years, having high yield, highly nutritious, selectively chosen as feed by animals, and can be grown in a wide variety of soil types. However, there are several negative properties which prevent this grass to be developed as planned. First of all, their flowers are very small while the pollens are short-lived which result in low level of seed formation. Moreover, the flowering period of each type of Napier grass is

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Received date : 04/04/06 Accepted date : 04/08/06

different making it more difficult and time-consuming to have them cross-pollinated. To alleviate this problem, tissue culture has been used as a tool to conserve and improve Napier grass cultivars. Different parts of Napier grass, i.e., leaf, seed, pollen, root have been cultured (Haydo and Vasil,1981 ; Rajasekaram *et al*.,1987). Selection of Napier grass cultured on 0-2% NaCl supplemented medium was also reported (Chandler and Vasil, 1984 ; Bajaj and Gupta, 1986). However, there is no report on the culturing of dwarf Napier grass or the induced mutation of this grass to get a salt tolerance cultivar. This project was aimed at finding a cultured medium formula to induce the young leaves of dwarf Napier grass into callus and also for the development of callus into plantlet. The ultimate goal, however, was to get a salt tolerant dwarf Napier grass to grow in the high saline soil which covered three-fourth of the waste land in the northeastern part of Thailand where animal feeding in cattle farm was greatly needed.

MATERIALS AND METHODS

Callus induction

Surface-sterilized young leaves of dwarf Napier grass (2 cm in size) were cross-sectioned to obtain approximately 0.25 cm in thickness. They were subject to culturing on MS medium supplemented with 5% coconut water and different amounts of 2,4-D at 0, 1, 2 and 3 mg/l. The culture was maintained in the dark for 15 days and transferred to the 16 h light per day for another 15 days. The percentage of callus formation as well as the callus types. i.e., friable callus, gelatinous callus and compact callus were recorded.

Plantlet induction

Calli were transferred to grow on the following four different plantlet induction media

1. MS + 5%coconut water
2. MS + 5%coconut water + 0.5 mg/l NAA + 1 mg/l BAP
3. MS + 5%coconut water + 0.5 mg/l NAA + 1 mg/l BAP + 0.5 mg/l 2,4-D
4. MS + 5%coconut water + 0.5 mg/l NAA + 0.5 mg/l BAP + 0.5 mg/l 2,4-D

The calli were cultured in the dark for four weeks. The percentage of green-spot production, root formation, shoot and complete plantlet were recorded.

Salt tolerant plant from irradiated callus in culture

Calli were irradiated with different doses of gamma radiation at 0, 10, 20, 30, 40 and 50 Gy and grown on the suitable plantlet induction medium. This selected medium was supplemented with NaCl at 0, 0.5, 1.0, 1.5 and 2.0%. The culture was kept for four weeks and the number of plantlets in each medium was recorded.

Field experiment

The plants survived from the gamma irradiation and salt supplemented in culture were transferred to the natural field at National Corn and Sorghum Research Center, Nakorn Rachasima province. The number of plants per clump, plant height, the width and length of leaves were recorded. Plants having good characters were selected for further experiment.

Salt tolerant plant in saline soil

Each selected clone of good characteristics was further propagated into five replicates and transferred to grow in the high salinity soil at Borabue district, Maha Sarakarm province for ten months. The salt tolerant plants were collected.

DNA fingerprinting of the selected good characteristic clones

The selected good characteristic clones of dwarf Napier grass grown at National Corn and Sorghum Research Center were genetically

determined using AFLP technique with 11 pairs of primers. The DNA fingerprinting patterns were analyzed.

RESULTS AND DISCUSSION

Callus induction

MS medium supplemented with 5% coconut water and 2 mg/l 2,4-D was found to give best result of dwarf Napier grass callus induction. This specific formula could induce as high as 95% callus formation (Table 1) and 67.5% of these calli was compact callus which was a preferred type of callus for plantlet induction. However, all three types of callus formation, i.e., friable callus, gelatinous callus, compact callus and also the mixed callus were found (Figure 1). This agreed with the results reported by Haydo and Vasil (1981) on the occurrence of three callus types of young leaf Napier grass culturing on MS medium supplemented with 0.5-1.0 mg/l 2,4-D.

Table 1 Percentage of callus and compact callus formation from young leaves cultured on MS medium supplemented with 5% coconut water, various concentration of 2,4-D for 15 days in the dark and 15 days light condition.

Concentrations of 2,4-D (mg/l)	No. of explants cultured	No. of explants of callus formation	Percent of callus formation	Percent of compact callus formation
0	20	0	0	0
0.5	20	0	0	0
1	36	16	44.4	41.6
2	40	38	95	67.5
3	23	23	100	26.1
4	32	32	100	40.75

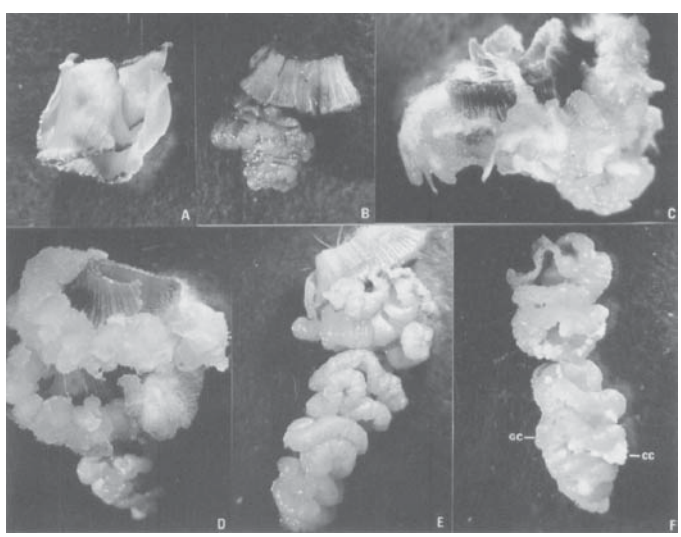

Figure 1 Different types of callus formation A) no callus formation, B) small callus, C) root formation on callus, D) friable callus, E) gelatinous callus and F) mixed callus. (GC=Gelatinous Callus, CC=Compact Callus)

Plantlet induction

Upon transferring compact calli to grow on MS medium supplemented with 5% coconut water and different amounts of NAA, BAP and 2,4-D, it was found that the MS medium supplemented with 5% coconut water containing 1 mg/l NAA, 0.5 mg/l BAP and 0.5 mg/l 2,4-D gave the highest average plantlet of 61.7% while those having only 0.5 mg/l NAA and 1 mg/l BAP gave a high average number of plantlet at 50.0%(Table 2). This result also corresponded to that of Wan and Vasil (1996) using similar formula of medium to induce embryonic callus from curled young leaf of Napier grass and further induced them to plantlet growth. Development of compact calli into plantlets are shown in Figure 2.

Irradiated callus in culture

Calli obtained from the MS medium containing 5% coconut water and 2 mg/l 2,4-D were irradiated with different doses of gamma radiation and transferred to grow on the most suitable plantlet induction medium of MS plus 5% coconut water supplemented with 0.5 mg/l 2,4-D, 1 mg/l NAA and 0.5 mg/l BAP with different percentage of NaCl for four weeks. The result showed that some calli irradiated at 10 and 20 Gy could survive in the medium containing 0.5 and 1.0% NaCl, while those exposed to gamma radiation up to 30 Gy could only sustain in the

Table 2 Percentage of plantlets formation from callus cultured on MS medium supplemented with 5% coconut water and various concentrations of 2,4-D, NAA and BAP for 4 weeks.

Concentrations of 2,4-D, NAA, BAP (mg/l)	No. of calli cultured	No. of calli developed into plantlets	Percent of plantlets formation
0:0:0	54	20	37.03
0:0.5:1	50	25	50.00
0.5:0.5:1	50	23	46.00
0.5:1:0.5	47	29	61.70

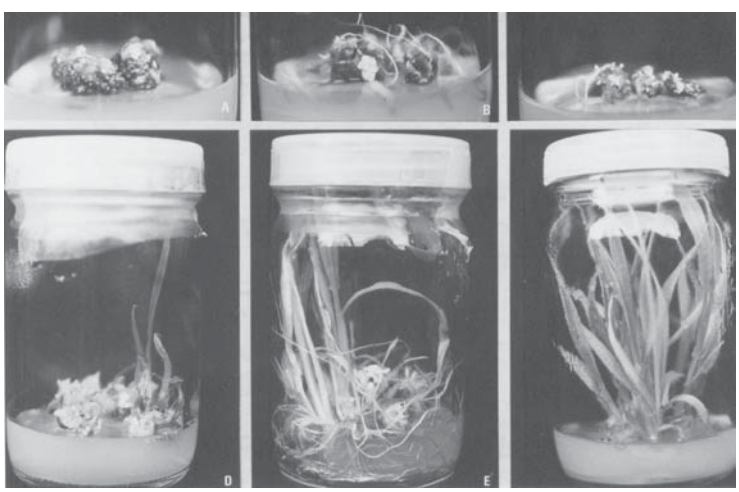

Figure 2 Development of callus into plantlets A) dead callus, B) root formation on callus, C) green spot formation on callus, D) shoot formation on callus, E) plantlet formation on callus and F) multiple shoot formation.

medium containing 0.5% NaCl (Table 3). However, the high salt media of 1.5 and 2.0% NaCl could not support any plantlet growth. By the same token, calli irradiated with high doses of gamma radiation at 40 and 50 Gy could not grow into plantlet in any of NaCl-added media.

Field experiment

All 85 irradiated clones of dwarf Napier grass survived in the cultured medium were transferred to grow in the natural field at National Corn and Sorghum Research Center, Nakorn Ratchasima province. Only 71 out of these 85 clones could survive in the field. However, another additional test was set to have all calli irradiated with 30 Gy and cultured on either 1% or 2% NaCl supplemented media. This second set of test resulted in additional 37 survival clones of plantlets grown in the field. The combined 108 clones of these two experimental sets were further grown in the natural field. These plants were cut at 30 days and their morphological characters, i.e., plant height, leaf width, fresh yield, percentage of crude protein(CP), percentage of acid detergent fiber (ADF) were recorded (Table 4). Twenty-one clones having desirable characters,i.e., high fresh yield and narrowed leaf type were selected.

The clones number 19 and 21 gave fresh yield/plant at 984.88 and 834.14 g, respectively, while clones number 7 and 8 contained the leaf width as low as 2.19 cm which was considered preferrable in making hay for animal feeding. As for percentage of crude protein, clone number 20 gave highest CP (20.06%) as the result of its short stem and bushy leaves compared to those of clone numbers 19 and 21 of somewhat lower CP (~16%). However, the percentages of acid detergent fiber (ADF) of these twenty-one selected clones were found to be in the close range of 26-30%

Salt tolerant plant in saline soil

Twenty-one selected clones of prospective salt tolerant plantlets were further propagated 5 times and transferred to grow in high salinity soil at Borabue district, Maha Sarakarm province for ten months. Eight out of these twenty-one clones could survive and the total of 11 plants were collected as salt tolerant dwarf Napier grass (Figure 3).

DNA fingerprinting analysis

DNA fingerprinting patterns of the selected twenty-one clones of dwarf Napier grass showed 1,048 bands. There were 993 different bands contributing to 94.75% polymorphism among these plants (Figure 4). Analysis of these bands using NTSYS-PC version 1.8 (Roklf, 1993) as formulated by Nei and Li (1997) gave the similarity index in the range of 0.569 to 0.784 (Table 5). Phylogenetic tree was constructed according to UPGMA method described by Sneath and Sokal (1973) as shown in Figure 5. It was distinctively seen that the control cultured sample of non-irradiated plant clearly separated itself from the group (sample#1) indicating that it was a progenitor of the others. When comparing the

Table 3 Number of plantlets from irradiated calli cultured on plantlet induction medium supplemented with various concentrations of NaCl for four weeks.

Concentration of NaCl (%)	No. of plantlets from irradiated calli at different radiation doses.						Total
	0 Gy	10 Gy	20 Gy	30 Gy	40 Gy	50 Gy	
0	7	20	7	7	12	10	54
0.5	5	17	2	1	0	0	25
1.0	0	5	1	0	0	0	6
1.5	0	0	0	0	0	0	0
2.0	0	0	0	0	0	0	0

Table 4 Leaf width, plant height, yield, % CP and %ADF of the selected dwarf Napier grass.

Clone No.	Radiation dose (Gy)	% NaCl	Leaf width (cm)	Plant height (cm)	Yield (g)	%CP	%ADF
1	0	0	2.76	120.46	540.57	17.19	28.72
2	0	0	2.53	124.50	825.20	15.28	28.99
3	0	0	2.9	118.50	800.12	15.31	27.82
4	10	0	2.57	112.50	273.29	17.49	26.58
5	10	0	2.25	112.50	388.04	18.11	26.55
6	10	0	2.52	111.00	546.23	16.08	30.16
7	10	0	2.19	120.75	377.84	15.59	29.69
8	10	0	2.19	78.00	134.08	17.07	27.21
9	10	0	2.53	117.50	360.25	15.76	27.46
10	10	0.5	2.28	79.50	199.27	17.24	26.48
11	10	0	2.36	106.00	202.70	16.40	26.00
12	20	0	2.93	125.00	658.90	16.04	29.72
13	20	0.5	2.41	127.25	496.39	16.68	29.82
14	30	0	2.51	120.25	603.90	16.29	27.21
15	40	0	2.71	122.50	663.55	16.83	28.81
16	40	0	2.43	103.25	235.51	16.74	29.40
17	40	0	2.46	120.00	508.51	17.88	25.99
18	40	0	2.25	111.50	276.72	14.84	29.02
19	40	0	2.65	135.00	984.88	16.85	28.80
20	40	0	2.79	110.75	257.26	20.06	28.07
21	50	0	2.75	129.50	834.14	16.31	26.29

Figure 3 Plantlets of dwarf Napier grass grown at Borabue district, Maha Sarakarm province A) at the beginning of the growth B) selected eight clones of salt tolerant plants after ten months of growth.

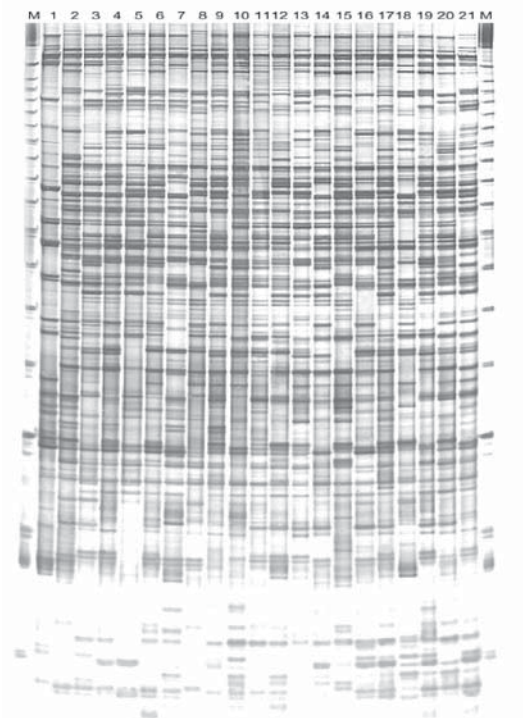

Figure 4 DNA fingerprinting patterns of 21 selected clones of dwarf Napier grass irradiated and grown in tissue culture. M is the standard DNA of 25 base pair ladder.

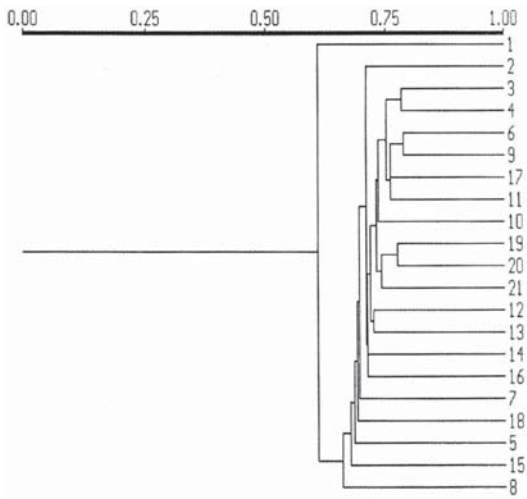

Figure 5 Phylogenetic tree showing the clusters of 21 selected irradiated dwarf Napier grass as analyzed by NTSYS-PC Version 1.8. Sample#1 was a non-irradiated plant.

similarity index between this control sample with the rest of the irradiated plants it was found to be 0.569-0.654 indicating that mutation at the DNA level had occurred more than 30% by random which also suggested that irradiation and tissue culturing were the main cause of genetic composition changes in these selected dwarf Napier grass.

CONCLUSION

The young leaves of "dwarf" Napier grass could be successfully cultured in the MS medium supplemented with 5% coconut water and 2 mg/l 2,4-D. Further transferring of highly compact type of callus to grow on MS medium containing 5% coconut water 1 mg/l NAA, 0.5 mg/l BAP and 0.5 mg/l 2,4-D could produce an average of 58.8% plantlet.

Irradiated calli at the level of 10 and 20 Gy could survive in the plantlet induction medium supplemented with 0.5 and 1.0% NaCL. After transferring the salt survival plantlets to the field for 30 days, those having superior characters, i.e., high fresh yield, narrow leaf, high crude protein (CP), high percentage of acid detergent fiber (ADF) were selected. Twenty-one clones were found to be qualified as potential clones of salt tolerance grass. DNA fingerprinting and similarity index of these 21 clones suggested that their morphological differences are likely contributed from the true mutation induced by irradiation and tissue culturing. The feasibility of having these dwarf Napier grass propagated in the high salinity soil of northeastern part of Thailand for animal feeding is quite promising.

Table 5 Similarity index of 21 selected clones of irradiated dwarf Napier grass using AFLP.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	1.000																				
2	0.654	1.000																			
3	0.650	0.738	1.000																		
4	0.623	0.721	0.784	1.000																	
5	0.616	0.679	0.710	0.718	1.000																
6	0.638	0.733	0.760	0.779	0.703	1.000															
7	0.593	0.695	0.718	0.723	0.657	0.714	1.000														
8	0.569	0.652	0.673	0.665	0.635	0.683	0.650	1.000													
9	0.595	0.718	0.742	0.760	0.703	0.786	0.710	0.686	1.000												
10	0.599	0.692	0.732	0.729	0.680	0.730	0.665	0.660	0.742	1.000											
11	0.615	0.707	0.726	0.752	0.682	0.744	0.696	0.664	0.774	0.750	1.000										
12	0.588	0.688	0.712	0.728	0.682	0.737	0.673	0.681	0.725	0.697	0.742	1.000									
13	0.601	0.690	0.716	0.725	0.669	0.718	0.689	0.652	0.726	0.708	0.740	0.724	1.000								
14	0.593	0.693	0.720	0.725	0.678	0.720	0.685	0.658	0.748	0.694	0.724	0.694	0.713	1.000							
15	0.596	0.680	0.662	0.695	0.654	0.706	0.662	0.650	0.684	0.672	0.673	0.673	0.659	0.665	1.000						
16	0.588	0.696	0.698	0.700	0.668	0.725	0.691	0.635	0.723	0.692	0.745	0.691	0.706	0.706	0.662	1.000					
17	0.604	0.723	0.746	0.753	0.706	0.765	0.705	0.661	0.756	0.729	0.760	0.739	0.756	0.719	0.725	0.729	1.000				
18	0.598	0.686	0.700	0.697	0.652	0.707	0.659	0.625	0.702	0.679	0.714	0.660	0.69	0.676	0.670	0.672	0.735	1.000			
19	0.623	0.727	0.741	0.746	0.682	0.750	0.713	0.677	0.751	0.735	0.753	0.718	0.739	0.732	0.678	0.726	0.766	0.719	1.000		
20	0.605	0.714	0.722	0.714	0.687	0.746	0.693	0.671	0.736	0.708	0.726	0.672	0.704	0.696	0.685	0.719	0.752	0.691	0.774	1.000	
21	0.608	0.699	0.713	0.712	0.682	0.716	0.655	0.638	0.715	0.691	0.714	0.672	0.692	0.674	0.679	0.694	0.739	0.678	0.746	0.740	1.000

ACKNOWLEDGEMENTS

This project was supported by the National Center for Genetic Engineering and Biotechnology.

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