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MORPHO-MOLECULAR CHARACTERIZATION OF NAPIER (*Pennisetum purpureum*) FODDER MUTANTS THROUGH SSR MARKERS

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ABSTRACT

Napier *(Pennisetum purpureum)* fodder is an important fodder supporting livestock systems in Bangladesh. Using gamma irradiation, we developed 48 Napier fodder mutants which were analyzed through morpho-molecular characterization. Among 48 Napier fodder mutants, 41 mutants were originated by applying radiation (20Gy, 30Gy, 40Gy and 50Gy) on them. The Napier mutants showed variations in survival rate and fresh weight. The survival rate (0-75%) was observed, 20Gy treated plants produced higher fresh weight in Napier-2, Napier-3, Rokona, Markiron grasses; whereas 30Gy treated plants produced higher fresh weight in Napier-1, Napier-4 and Pakchong. The total of the alleles found in molecular characterization were 17 and the mean number of allele per locus was 3.4. The PIC value ranged from 0.3047 to 0.6587 having an average of 0.4704 per locus. The highest gene diversity (0.7101) was observed in primer Xipes0093, and the lowest gene diversity (0.3750) was observed in primer PSMP2255, having an average diversity of 0.5286. Pair wise genetic distance values ranged (0.0-1.0) with highest between Napier-2 40Gy vs (Napier-1control, Napier-2 20Gy Napier-2 30Gy), Napier -3 control, vs (Napier-1control, Napier-2 20Gy Napier-2 30Gy), Napier- 3 20Gy vs (Napier-1control, Napier-2 20Gy Napier-2 30Gy) ,(Napier-3 20Gy, Napier-3 30Gy Napier-1 20Gy, Napier-3 50Gy) vs (Napier -2 20Gy, Napier-3 control, Napier-3 20Gy) etc. The UPGMA displayed five major clusters including sub-clusters. Cluster 5 included the highest number of genotypes (21) and cluster 3 included the lowest number of genotypes (2). The results may be useful for future breeding program for Napier fodder development.

Keywords: Gamma Irradiation, Fodder mutants, SSR-Markers, Morpho-Molecular Characterization, Gene Diversity.

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INTRODUCTION

Feed shortage is the major reason for low productivity of livestock in Bangladesh. Approximately 8% of total protein for human consumption comes from livestock. Growing forage with a high yield potential and good quality is important to overcome limited supply of roughage during annual drought and reduce the amounts of concentrate needed for cattle farming in the tropics (Kamal et al., 2016). The demand of fodder production is increasing today because there is a limited listed livestock feed resource in the country. Animal in Bangladesh mainly survives on the common local grasses that are not available throughout the year. Developing of different high yielding fodder variety that it will be able to give a better nutrition to the livestock. The country quantitively requires 49 million tons DM of roughage and 24 million tons of concentrate in a year (Huque et al., 2014) and meets only 56.4% and 20%, respectively of their requirements.

Napier grass or "elephant grass" or "Sudan grass" which is scientifically known as *Pennisetum purpureum* grass, can produce lots of high-protein forage. It is a deep-rooted high yielding perennial bunch that is native to eastern and central Africa (Boon man, 1993) and grows rapidly in Southeast Asia also. It grows in tropical and sub-tropical regions with a wide range of annual moisture from 750 to 2,500 mm rainfall and in altitudes ranging from sea level to altitudes of over 2100 m, but frost appears to limit its cultivation above this altitude (Skermanand Riveros, 1990). Napier is a perennial grass and its excellent characteristics like drought tolerance, wide range of soil conditions, high photosynthetic and water-use efficiency (Anderson et al., 2008), now it is regarded as the forage of choice not only in the tropics but also worldwide (Hanna et al., 2004). Though it is rain friendly but due to its drought-tolerant, it can also grow well in drier areas. It does not grow well in waterlogged areas. It can be grown along with fodder trees field boundaries or along contour lines or terrace risers to help control erosion. It can be intercropped with crops such as legumes and fodder trees, or as a pure stand.

Mutation breeding is one of the oldest methods in plant breeding to generate mutants with desirable traits. Physical mutagens such as gamma irradiation are widely used to produce mutants in different crops. Gamma irradiation has also succeeded in forage grasses and turf grasses. Gamma irradiation used in seeds and other planting materials such as cuttings, pollen, or tissue cultured Calli (Beyaz and Yildi, 2017).

Morphological markers were the earliest used in germplasm management and were fully exploited in Mendelian era. Molecular markers do not have such limitations and can be used to detect variation at the DNA level and have proven to be effective tools for distinguishing between closely related genotypes (Edwards et al., 1991). It’s also essential for mapping genes of interest, marker-assisted breeding, and cloning genes using mapping-based cloning strategies (Hayashi et al., 2004). The use of molecular markers for tracking of loci and genome regions is now prominent among crop breeders with a large number of molecular markers linked to disease resistance traits available in most major crop species (Jain et al., 2002; Gupta and Varshney, 2004).

Among these available molecular markers, SSR is one of the most widely used microsatellite marker which also known as microsatellites DNA marker. In comparison to multi-locus markers, microsatellites have the advantage of their locus specificity, co-dominant nature, high polymorphism, and reproducibility (Powell et al., 1996). In addition, their detection can be easily automated (Hernandez et al., 2002).

In the current study, SSR markers were used to assess the genetic diversity of Napier fodder mutants. The specific objectives of the study were to identify the effect of gamma-ray irradiation on Napier fodder crops, detect the genetic variation and determine genetic relationship among Napier fodder mutants.

MATERIALS AND METHODS

Gamma Irradiated for Napier fodder

Seven Napier cultivars namely: Napier-1, Napier-2, Napier-3, Napier-4, Rokona, Pakchong and Markiron grasses were collected from Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka. Twenty-eight double node cuttings from each cultivar were irradiated in different dozes (20Gy, 30Gy, 40Gy and 50Gy) using ^{60}Co source in Bangladesh Institute of Nuclear Agriculture (BINA). Control node cuttings were not irradiated. These irradiated cuttings were transplanted at 60cm x 60cm plant to plant and row to row distance along with control. Irrigation and fertilizer were applied as and when necessary.

DNA amplification with microsatellite markers and gel electrophoresis

The primers which were previously developed for pearl millet (Allouis et al., 2001; Budak et al., 2003; Mariac et al., 2006) and demonstrated to be transferable to Napier fodder mutants (Azevedo et al., 2012) were used for genotyping (Table 1).

Table 1. List of primers for PCR (finger printing) analysis of Napier genotypes.

Sl. No.	Primer Name	Sequence	Base	Annealing Temp. (^{0}C)
1	CTM10_F	GAGGCAAAAGTGGAAGACAG	20	53
	CTM10_R	TTGATTCCCGGTTCTATCGA	20	
2	CTM12_F	GTTGCAAGCAGGAGTAGATCGA	22	52
	CTM12_R	CGCTCTGTAGGTTGAACTCCTT	22	
3	PGIRD25_F	CGGAGCTCCTATCATTCCAA	20	54
	PGIRD25_R	GCAAGCCACAAGCCTATCTC	20	
4	PSMP2255_F	CATCTAAACACAACCAATCTTGAA	25	52
	PSMP2255_R	C TGGCACTCTTAAATTGACGCAT	22	
5	Xipes0093_F	GGATCTGCAGGTTTGGACAT	20	52
	Xipes0093_R	CCAAGCACTGAAACATGCAC	20	

Genomic DNA Isolation

Genomic DNA was extracted from the collected samples following the cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987), at the Laboratory of Biotechnology division, BINA, Mymensingh. The PCR conditions were as initial denaturation at 94ºC for 5 min, 35 cycles denaturation at 94ºC for 45 seconds, annealing at respective temperature of individual primer for 1 min, Polymerization at 72ºC for 1 min, repeating steps 2-4 for 35 times and incubation at 72ºC for 5 minutes.

Data scoring and analysis

The statistics including the number of alleles per locus, major allele frequency, gene diversity and PIC values were determined using POWER MARKER version 3.23 (Liu and Muse, 2005), a genetic analysis software. Molecular weights for microsatellite products, in base-pairs, were estimated with Alpha Ease 4C software. The individual fragments were assigned as alleles of the appropriate microsatellite loci. The PIC or expected heterozygosity for each SSR marker was calculated based on the formula $Hn= 1-Pi^2$, where Pi is the allele frequency for the i^{th} allele (Nei et al., 1983). The 48 genotypes were clustered based on the matrix of genetic similarities using UPGMA. The cluster analysis and dendogram construction were performed with NTSYS-PC (version 2.1).

RESULTS AND DISCUSSION

Morphological characterization of 48 Napier fodder genotypes

We observed survival percentage varied due to the effect of different dose of gamma rays. It ranged from (0-75%) and the control has the highest survival rate (75% in Napier-1 control). Among the mutants, highest survival rate has recorded in Napier-1 20Gy (64%), Napier-1 30Gy (57%), Napier-3 30Gy (53%) and Napier-4 30Gy (50%). Most of the cuttings didn't survive in higher radiation dose like 40Gy and 50Gy (Table 2). Variations were observed in all the doses of gamma irradiations. The likelihood to get putative and desirable mutants in 20Gy, 30Gy and 40Gy of Gamma rays; but some bushy and dwarf type mutants could be found in 50Gy as well. It was found that out of 7 fodder cultivars, 20Gy treated plants produced higher fresh weight in Napier-2, Napier-3, Rokona, Markiron grasses; whereas 30Gy treated plants produced higher fresh weight in Napier-1, Napier-4 and Pakchong. They produced higher fresh weight than those of control plants (parents) except in few cases (Table 3).

Table 2. Effect of Gamma radiation dose on survival of different fodder crops

Name of fodder	Radiation dose (Gy)	No. cutting irradiated	Cutting survived	Survival (%)
Napier-1	20	28	18	64
	30	28	16	57
	40	28	06	21
	50	28	04	14
	Control	28	21	75
Napier-2	20	28	11	39
	30	28	06	21
	40	28	07	25
	50	28	05	17
	Control	28	16	57
Napier-3	20	28	07	25
	30	28	15	53
	40	28	07	25
	50	28	03	10
	Control	28	14	50
Napier-4	20	28	00	00
	30	28	14	50
	40	28	04	14
	50	28	00	00
	Control	28	17	60
Rokona	20	28	08	28
	30	28	03	10
	40	28	00	00
	50	28	00	00
	Control	28	05	17
Pakchong	20	28	04	14
	30	28	01	04
	40	28	01	04
	50	28	00	00
	Control	28	05	17
Markiron	20	28	11	39
	30	28	03	10
	40	28	00	00
	50	28	00	00
	Control	28	06	21

Table 3. Effect of radiation in fresh weight (Mean±SE) of fodders in three different cutting

Name of fodder	Radiation dose (Gy)	1st cutting fresh wt. (g)	2nd Cutting fresh wt. (g)	3rd cutting fresh wt. (g)
Napier-1	20	664±52	864±54	1422±110
	30	1106±90	1250±50	2678±125
	40	620±30	810±54	1050±63
	50	248±20	400±43	640±55
	Control	1044±60	1170±76	2460±170
Napier-2	20	1860±75	1569±86	3686±123
	30	988±65	1280±55	1752±58
	40	470±30	685±38	1760±74
	50	366±30	589±45	380±23
	Control	3475±150	3070±124	3052±110
Napier-3	20	3206±122	3480±134	6440±189
	30	2260±105	2170±97	4480±112
	40	1048±75	876±57	4690±196
	50	478±35	654±45	4100±156
	Control	4248±250	3900±215	5520±210
Napier-4	20	-	-	-
	30	1600±85	1400±77	3674±185
	40	715±45	870±65	3500±153
	50	-	-	
	Control	1300±60	1250±75	2336±120
Rokona	20	2498±126	2800±154	3838±223
	30	875±25	750±33	1510±61
	40	-	-	-
	50	-	-	-
	Control	1047±54	1250±65	1386±62
Pakchong	20	643±27	860±35	1860±72
	30	1100±55	1380±64	2275±78
	40	200±25	245±32	525±41
	50	-	-	
	Control	636±35	867±44	1430±54

Name of fodder	Radiation dose (Gy)	1st cutting fresh wt. (g)	2nd Cutting fresh wt. (g)	3rd cutting fresh wt. (g)
Markiron	20	587±53	878±65	2144±78
	30	450±45	540±65	860±62
	40	-	-	-
	50	-	-	-
	Control	91.67	7.36	36.05

These present studies reported that the survival rate of the fodder is varied compare to the doses of irradiation like Napier-1 20Gy (64%), Napier-1 30Gy (57%) and Napier-3 30Gy (53%) have better survival rate compare with their controls. On the other hand, fresh weight of Napier fodder has the effect on radiation doses, the highest fresh weight was recorded 20Gy, 30Gy (expect Napier-4 20Gy) and the lowest was 40Gy and 50Gy doses, some bushy and dwarf type mutants could be found. Mutlu et al. (2015) performed on experiment that Bermuda grass irradiated with 70, 90 or 110Gy using a ^{60}Co source to measure and determine variations in morphologically and characteristically. Survival rates of stolons exposed to 70, 90 and 110Gy were 76%, 43% and 17% respectively, 6 weeks after treatment. Dosages of 85 and 57Gy were determined as LD_{50} and LD_{20} for the cuttings, respectively. A total of four mutant lines (0.3 % of the irradiated plants) showed a distinct dwarfed growth habit. Three of these lines were originated from 70Gy and one from 110Gy. These mutant lines exhibited more dwarf growth habit, higher shoot density, finer leaf texture than parental genotype. It was observed in both studies that higher the doses of radiation lower the rate of survival and dwarfed growth habit.

Molecular characterization of 48 Napier fodder genotypes

Five SSR loci were used to study diversity in 48 Napier fodder genotypes. The results that were obtained from the experiments using microsatellite markers on 48 Napier crops are mentioned below (Fig. 1- 3)

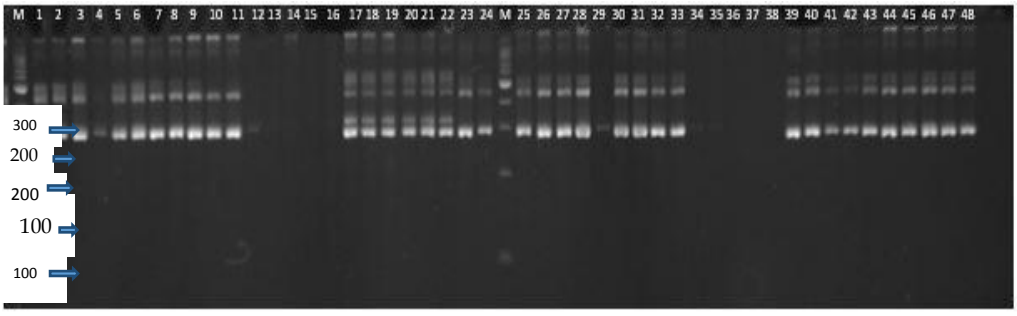

Figure 1. Microsatellite profile of 48 Napier genotypes (41 mutants and 7 controls) at locus CTM 12

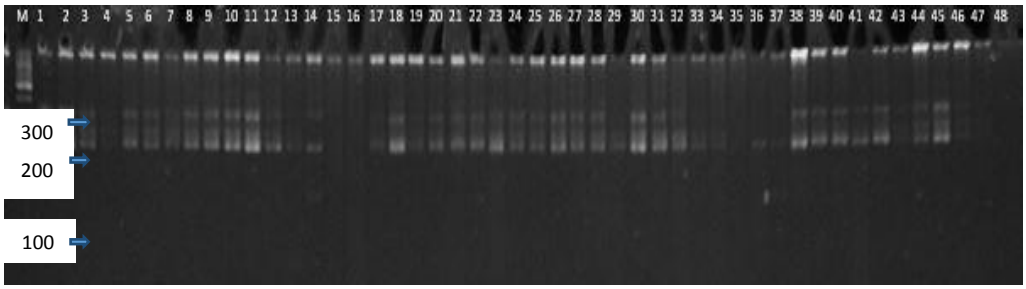

Figure 2. Microsatellite profile of 48 Napier genotypes (41 mutants and 7 controls) at locus PSMP2255

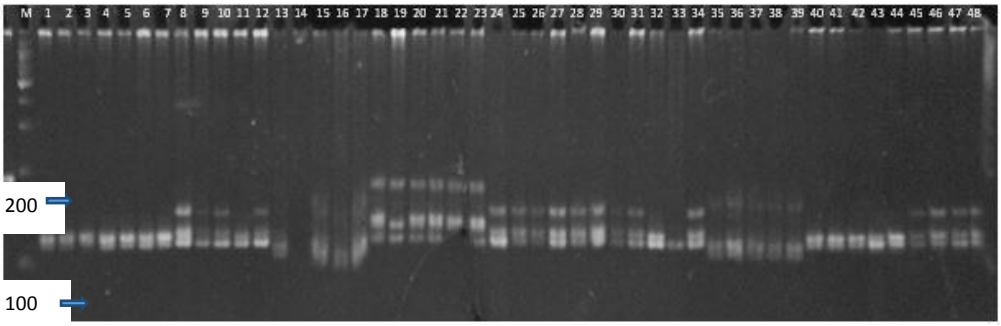

Figure 3. Microsatellite profile of 48 Napier genotypes (41 mutants and 7 controls) at locus Xipes0093

Here, M= 100bp ladder, 1= Napier- 1 control , 2= Napier- 1 20Gy, 3= Napier- 1 20Gy, 4= Napier - 1 30Gy, 5= Napier-1 30Gy, 6= Napier-1 40Gy, 7= Napier-1 50Gy, 8= Napier-2 control, 9=Napier-2 20Gy, 10= Napier-2 20Gy, 11 = Napier -2 30Gy, 12= Napier-2 30Gy, 13= Napier-2 40Gy, 14= Napier-2 50Gy, 15= Napier-3 control, 16= Napier-3 20Gy, 17= Napier-3 20Gy, 18= Napier-3 30Gy,19= Napier-3 30Gy, 20= Napier-3 40Gy, 21= Napier-3 50Gy, 22= Napier-4 control, 23= Napier-4 20Gym , 24= Napier-4 20Gy, 25= Napier-4 30Gy, 26= Napier-4 30Gy, 27= Napier-4 40Gy, 28= Napier-4 50Gy, 29= Rokona– control, 30= Rokona- 20Gy, 31= Rokona- 20Gy, 32= Rokona- 30Gy, 33= Rokona- 30Gy, 34= Rokona- 40Gy, 35= Rokona- 50Gy, 36= Pakchong- control, 37= Pakchong- 20Gy, 38= Pakchong- 20Gy, 39= Pakchong- 30Gy, 40-Pakchong- 30Gy , 41-Pakchong- 40Gy, 42-Pakchong- 50Gy , 43-Markiron– control, 44=Markiron- 20Gy, 45= Markiron- 20Gy , 46=Markiron-30Gy, 47=Markiron- 40Gy, 48=Markiron- 50Gy

Size of alleles

In case of PGIRD25, allele size was 160-168bp, CTM 10, CTM 12, PSMP2255, Xipes0093 displayed the range 163-202bp. 294-296bp, 218bp, 110-116bp, respectively (Table 4).

Major allele frequency

The allele with the highest frequency is termed as major allele or most common allele at each locus can be defined as major allele, among all mutants, on an average 61% of them shared a common major allele ranging from 40% (Xipes0093) to 75% (PSMP2255) at each locus (Table 4).

Gene diversity

The highest gene diversity (0.7101) was observed in Xipes0093 and the lowest gene diversity (0.3750) was observed in PSMP2255, having an average diversity of 0.5286. It was found that marker detecting the higher number of alleles showed higher gene diversity, on the other hand lower number of alleles expressed lower gene diversity (Table 4).

PIC value

Polymorphism information content (PIC) value is a reflection of allele diversity and frequency among the mutants. PIC value of each marker can be determined on the basis of its allele. PIC varied significantly for all the studied SSR loci. In this study, the level of polymorphism among 48 Napier fodder mutants was evaluated by calculating PIC values for each of the 5 SSR loci. The highest PIC values ranged from 0.3047 to 0.6587 having an average of 0.4704 per locus. The highest PIC value was 0.6587 for Xipes0093 and the lowest was 0.3047 for PSMP225 and (Table 4).

Table 4. Data of allele size, major allele frequency, allele number, heterozygosity, gene diversity and PIC value found among 48 Napier genotypes (41 mutants and 7 control) for five SSR markers.

Marker	Allele Size (bp)	Major Allele Frequency (%)	Allele No	Heterozygocity	Gene Diversity	PIC
PGIRD25	160-168	69	4	0.0000	0.4783	0.4314
CTM -10	163-202	56	3	0.0000	0.5877	0.5224
CTM -12	294-296	67	3	0.0000	0.4922	0.4347
PSMP2255	218	75	2	0.0000	0.3750	0.3047
Xipes0093	110-116	40	5	0.0000	0.7101	0.6587
Mean		61	3.4	0.0000	0.5286	0.4704

Based on SSR marker 48 Napier genotypes analysis using UPGMA

Dendogram based on Nei's (1983) genetic distance using UPGMA indicated differentiation of the 48 Napier mutants by five SSR markers. The UPGMA cluster analysis led to the grouping of 48 Napier genotypes in five major clusters at 60% cut off. (Fig. 4). In Cluster-1, ten mutants namely: Napier-2 40Gy, Napier-3 control, Napier-3 20Gy, Pakchong-control, Rokona-40Gy, Rokona-50Gy, Napier-2 30Gy, Napier-2 50Gy, Pakchong-20Gy, Pakchong-20Gy clustered together and they were

grouped in same category in molecular analysis. Cluster-2 consisted of eleven mutants of Napier namely Napier-2 20Gy, Napier-2 20Gy, Napier-2 30Gy, Napier-1 30Gy, Napier-2 control, Napier- 1 control, Napier- 1 20Gy, Napier-1 20Gy, Napier-1 30Gy, Napier-1 40Gy, Napier-1 50Gy. Cluster-3 comprised of two mutants of Napier and they were Markiron- 20Gy, Markiron- 40Gy. Cluster-4 consisted of four mutants of Napier namely: Markiron- control, Napier-3 30Gy, Markiron- 30Gy, Markiron - 50Gy. Cluster-5 showed 2 sub clusters (5A, 5B), sub cluster 5A comprised of six mutants namely: Pakchong- 30Gy, Pakchong 40Gy, Rokona- 30Gy, Pakchong- 30Gy, Pakchong- 50Gy, Markiron- 20Gy. Sub cluster 5B contained fifteen mutants Napier-4 20Gy, Napier-4 20Gy, Rokona- 20Gy, Rokona- 20Gy, Rokona- 30Gy, Napier-3 20Gy, Napier-3 30Gy, Napier-3 40Gy, Napier-3 50Gy, Napier-4 control, Napier-4 30Gy, Napier-4 30Gy, Napier-4 40Gy, Napier-4 50Gy, Rokona–control.

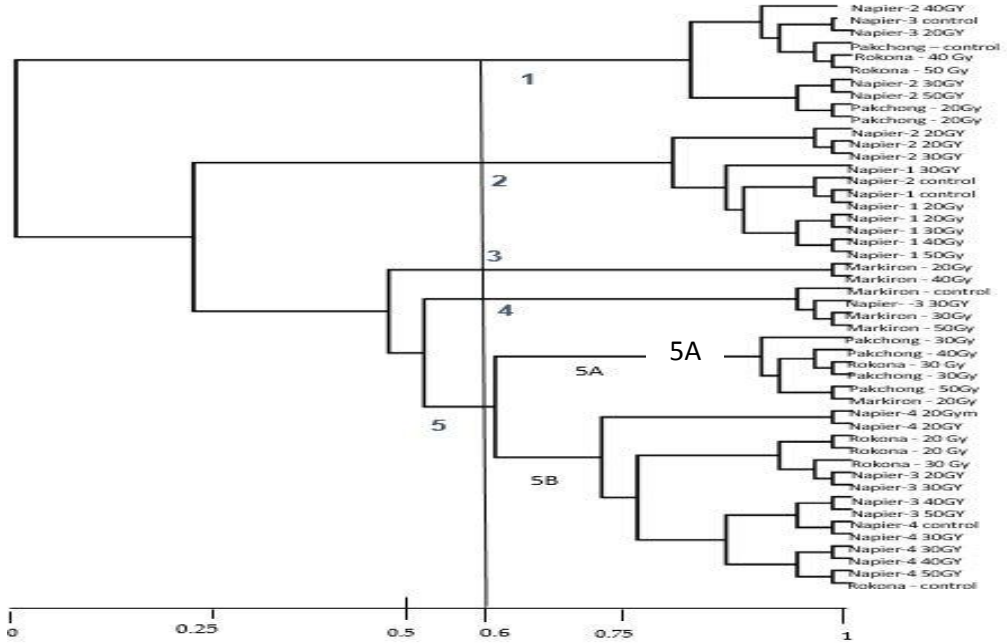

Figure 4. UPGMA Dendogram of 48 Napier fodder genotypes based on Nei's Genetic Distance Genetic characterization of cultivars is an important step in any breeding programme for selection of appropriate parental lines (Zhang et. al., 2009). Several DNA marker systems for germplasm genetic characterization are available but SSRs have been found most adequate in detecting relationships among populations as well as obtaining specific genetic fingerprints.

In order to detect the genetic diversity of Napier genotypes, PCR based microsatellite marker analysis was done. There are a total of 17 alleles amplified from 5 loci of Napier fodder genotype (*Pennisetum purpureum*) ranging from 1 to 3. The average number of alleles per locus was 3.4 (Table 5); Negawo et al. (2018) conducted an experiment with 171 genotypes and 20 microsatellite primer pairs they reported that The number of alleles for the EMBRAPA active gene bank collection, and the ILRI gene bank collection per marker ranged from 1 to 15 and 1 to 22 with averages of 4.65 and 7. Both collections (EMBRAPA and ILRI) were combined and noted that the number of alleles per marker ranged from 1 to 23 with an average of 7.45, which is higher than this present study. These differences might be due to different genotypes of Napier, geographical environment, different microsatellite markers and different experimental set up.

The genetic distances and genetic identity were computed by Nei's (1983) genetic distance. Genetic identity, which estimated the proportion of genes that were identical in two mutants and genetic distance which estimated the proportion changed gene that had occurred in the separate evolution of two mutants. The higher is the genetic distance; the lower is the genetic identity. Results of pair wise comparison showed the highest genetic distance (1.0) between Napier-2 40Gyvs (Napier-1control, Napier-2 20Gy Napier-2 30Gy), Napier-3 control, vs (Napier-1control, Napier-2 20Gy Napier-2 30Gy), Napier-3 20Gy vs (Napier-1control, Napier-2 20Gy Napier-2 30Gy), (Napier-3 20Gy, Napier-3 30Gy Napier-1 20Gy, Napier-3 50Gy) vs (Napier-2 20Gy, Napier-3 control, Napier-3 20Gy) etc. and the lowest genetic distance value (0.0) was observed in Napier-2 30Gy vs Napier-2 20Gy, Napier-2 50Gy vs Napier-2 30Gy, Napier-1 20Gy vs Napier-1 Control, (Napier-3 40Gy, Napier-3 50Gy Napier-4 control vs Napier-3 20Gy, Napier-3 30Gy, Napier-3 30Gy) (Table 5) A genetic distance of zero means that there are no differences in the two results and there is an exact match. Okukenu et al. (2020) showed that the population of *P. purpureum* are closely related and have a recent ancestor. The results of Nei's genetic distance measurement of the nine populations as collected from the nine locations showed that there was a phylogenetic relationship between the *P. purpureum* populations, considering a population as clones from the same place of collection.

Table 5. Summary of genetic distance values among 48 Napier genotypes using five SSR markers (1st part)

Gen	1	10	11	12	13	14	15	16	17	18	19	2	20	21	22	23	24	25	26	27	28	29	3	30	31
1	0.00																								
10	0.40	0.00																							
11	0.40	0.00	0.00																						
12	0.80	0.80	0.80	0.00																					
13	1.00	1.00	1.00	0.40	0.00																				
14	0.80	0.80	0.80	0.00	0.40	0.00																			
15	1.00	1.00	1.00	0.20	0.20	0.20	0.00																		
16	1.00	1.00	1.00	0.20	0.20	0.20	0.00	0.00																	
17	0.40	0.40	0.40	0.80	1.00	0.80	1.00	1.00	0.00																
18	0.40	0.40	0.40	0.80	1.00	0.80	1.00	1.00	0.00	0.00															
19	0.60	0.60	0.60	1.00	0.80	1.00	0.80	0.80	0.20	0.20	0.00														
2	0.00	0.40	0.40	0.80	1.00	0.80	1.00	1.00	0.40	0.40	0.60	0.00													
20	0.40	0.40	0.40	0.80	1.00	0.80	1.00	1.00	0.00	0.00	0.20	0.40	0.00												
21	0.40	0.40	0.40	0.80	1.00	0.80	1.00	1.00	0.00	0.00	0.20	0.40	0.00	0.00											
22	0.40	0.40	0.40	0.80	1.00	0.80	1.00	1.00	0.00	0.00	0.20	0.40	0.00	0.00	0.00										
23	0.60	0.60	0.60	0.80	1.00	0.80	1.00	1.00	0.20	0.20	0.40	0.60	0.20	0.20	0.20	0.00									
24	0.60	0.60	0.60	0.80	1.00	0.80	1.00	1.00	0.20	0.20	0.40	0.60	0.20	0.20	0.20	0.00	0.00								
25	0.40	0.40	0.40	0.80	1.00	0.80	1.00	1.00	0.00	0.00	0.20	0.40	0.00	0.00	0.00	0.20	0.20	0.00							
26	0.40	0.40	0.40	0.80	1.00	0.80	1.00	1.00	0.00	0.00	0.20	0.40	0.00	0.00	0.00	0.20	0.20	0.00	0.00						
27	0.40	0.40	0.40	0.80	1.00	0.80	1.00	1.00	0.00	0.00	0.20	0.40	0.00	0.00	0.00	0.20	0.20	0.00	0.00	0.00					
28	0.40	0.40	0.40	0.80	1.00	0.80	1.00	1.00	0.40	0.40	0.20	0.40	0.00	0.00	0.00	0.20	0.20	0.00	0.00	0.00	0.00				
29	0.40	0.40	0.40	0.80	1.00	0.80	1.00	1.00	0.00	0.00	0.20	0.40	0.00	0.00	0.00	0.20	0.20	0.00	0.00	0.00	0.00	0.00			
3	0.00	0.40	0.40	0.80	1.00	0.80	1.00	1.00	0.40	0.40	0.60	0.00	0.40	0.40	0.40	0.60	0.60	0.40	0.40	0.40	0.40	0.40	0.00		
30	0.40	0.40	0.40	0.80	1.00	0.80	1.00	1.00	0.00	0.00	0.20	0.40	0.00	0.00	0.00	0.20	0.20	0.00	0.00	0.00	0.00	0.00	0.40	0.00	
31	0.40	0.40	0.40	0.80	1.00	0.80	1.00	1.00	0.00	0.00	0.20	0.40	0.00	0.00	0.00	0.20	0.20	0.00	0.00	0.00	0.00	0.00	0.40	0.00	0.00

Here, 1= Napier-1 control , 2= Napier-1 20Gy, 3= Napier-1 20Gy, 4= Napier-1 30Gy, 5= Napier-1 30Gy, 6= Napier-1 40Gy, 7= Napier-1 50Gy, 8= Napier-2 control, 9=Napier-2 20GY, 10= Napier-2 20GY, 11 = Napier -2 30GY, 12= Napier-2 30GY, 13= Napier-2 40GY, 14= Napier-2 50GY, 15= Napier-3 control, 16= Napier-3 20GY, 17= Napier-3 20GY, 18= Napier-3 30GY,19= Napier-3 30GY, 20= Napier-3 40GY, 21= Napier-3 50GY, 22= Napier-4 control, 23= Napier-4 20Gym , 24= Napier-4 20GY, 25= Napier-4 30GY, 26= Napier-4 30GY, 27= Napier-4 40GY, 28= Napier-4 50GY, 29= Rokona – control, 30= Rokona - 20 Gy, 31= Rokona - 20 Gy, 32= Rokona - 30 Gy, 33= Rokona - 30 Gy, 34= Rokona - 40 Gy, 35= Rokona - 50 Gy, 36= Pakchong – control, 37= Pakchong - 20Gy, 38= Pakchong - 20Gy, 39= Pakchong - 30Gy, 40-Pakchong - 30Gy , 41-Pakchong - 40Gy, 42-Pakchong - 50Gy , 43-Markiron – control , 44= Markiron - 20Gy, 45= Markiron - 20Gy , 46= Markiron - 30Gy, 47= Markiron - 40Gy, 48= Markiron - 50Gy

Gen*= Genotype used in this Study.

The UPGMA dendogram showed that Napier-2 40Gy, Napier-3 control, Napier-3 20Gy, Pakchong– control, Rokona - 40Gy, Rokona - 50Gy, Napier-2 30Gy, Napier-2 50Gy, Pakchong - 20Gy, Pakchong - 20Gy clustered together and they were grouped in same cluster (cluster 1). Napier-2 20Gy, Napier-2 20Gy, Napier -2 30Gy, Napier - 1 30Gy, Napier-2 control, Napier-1 control, Napier-1 20Gy, Napier-1 20Gy, Napier-1 30Gy, Napier-1 40Gy, Napier-1 50Gy were grouped into same cluster (cluster-2). Markiron- 20Gy, Markiron - 40Gy were clustered in cluster-3. Markiron– control, Napier-3 30Gy, Markiron- 30Gy, Markiron- 50Gy were clustered in cluster-4. Other 21 mutants formed another cluster named cluster 5. Similarly, Negawo et al. (2018) experiments showed that the hierarchical cluster analysis assembled the collections into 4 main clusters with further sub clusters. It was observed in both studies that genetically related genotypes were grouped into same cluster due to their similarity in genetic information and lower genetic distance. Higher genetic distance and lower genetic similarity were remained between one cluster to another.

CONCLUSION

Radiation has been used successfully to bring genetic variability in different crop species and it is considered as a useful tool for improvement of different crop plants. Mutation breeding is now widely used for inducing genetic changes and creation of new genetic resources, particularly in crops that are not easily amenable to improvement through conventional technique. Survival rate and fresh weight is an important parameter in the productivity of forage crops. Survival rate and the fresh weight of the Napier fodder genotypes were affected by the doses of irradiation. According to the dendogram and genetic distance it could be concluded that the higher genetic distance indicated the lower genetic identity. On the contrary, the lower genetic distance indicated the high value of genetic identity. The polymorphism was detected among the genotypes can be used in breeding program to maximize the use of genetic resources as well as to develop Napier mutants. The mutant pairs which showed genetic distance can be used in further breeding program.

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REFERENCES

Allouis, S., Qi, X., Lindup, S., Gale, M.D. and Devos, K.M. (2001). Construction of a BAC library of pearl millet, *Pennisetum glaucum*. *Theoretical and Applied Genetics*, 102(8): 1200-1205.

Anderson W.F., Dien B.S., Brandon S.K. and Peterson J.D. (2008). Assessment of Bermuda grass and bunch grasses as feedstock for conversion to ethanol. *Applied Biochemistry and Biotechnology*, 145: 13-21.

Azevedo, A.L.S., Costa, P.P., Machado, J.C., Machado, M.A., Vander Pereira, A. and da Silva Lédo, F.J. (2012). Cross species amplification of *Pennisetum glaucum* microsatellite markers in *Pennisetum purpureum* and genetic diversity of Napier grass accessions. *Crop Science*, 52(4): 1776-1785.

Beyaz, R. and Yildiz, M. (2017). The use of gamma irradiation in plant mutation breeding. *Plant Engineering*, 33-46.

Boonman, J.G. (1993). East African grasses and fodders: Their ecology and husbandry. Kluwer academic Publishers, Dortrecht, Netherland. 343.

Budak, H., Pedraza, F., Cregan, P.B., Baenziger, P.S. and Dweikat, I. (2003). Development and utilization of SSRs to estimate the degree of genetic relationships in a collection of pearl millet germplasm. *Crop Science*, 43(6): 2284-2290.

Doyle, J.J. and Doyle, J.L. (1990). Isolation of plant DNA from fresh tissue. Focus 12 13-15.

Edwards, A., Civitello, A., Hammond, H.A. and Caskey, C.T. (1991). DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. *American journal of human genetics*, 49(4): 746-756.

Gupta, P.K. and Varshney, R.K. (2004). Cereal genomics: an overview. Cereal genomics, 1-18.

Hanna W.W., Chaparro C.J., Mathews B.W., Burns J.C., Sollenberger L.E. and Carpenter J.R. (2004). Perennial Pennisetums. In: Moser LE, Burson BL and Sollenberger LE (eds) Warm season (C4) Grasses. *American Society of Agronomy Monograph*. 503-535.

Hayashi, K., Hashimoto, N., Daigen, M. and Ashikawa, I. (2004). Development of PCR-based SNP markers for rice blast resistance genes at the Piz locus. *Theoretical and Applied Genetics*, 108(7): 1212-1220.

Hernández, P., Laurie, D.A., Martin, A. and Snape, J.W. (2002). Utility of barley and wheat simple sequence repeat (SSR) markers for genetic analysis of Hordeumchilense and tritordeum. *Theoretical and Applied Genetics*, 104(4), 735-739.

Huque, K.S. and Sarker, N.R. (2014). Feeds and feeding of livestock in Bangladesh: performance, constraints and options forward. *Bangladesh Journal of Animal Science*, 43(1): 1-10.

Jain, S.M., Brar, D.S. and Ahloowalia, B.S. (Eds.). (2010). Molecular techniques in crop improvement. New York, NY, USA: Springer. IX, 772.

Kamal, M.T., Hashem, M.A., Sarker, N.R., Jahan, R. and Mujaffar, M. (2016). Effects of organic manure on production performance and nutritive values of different Napier cultivars. *Journal of the Bangladesh Society for Agricultural Science and Technology*, 13(1&2):1-4.

Liu, K. and Muse, S.V. (2005). Power Marker: an integrated analysis environment for genetic marker analysis. *Bioinformatics*, 21: 2128-2129.

Mariac, C., Luong, V., Kapran, I., Mamadou, A., Sagnard, F., Deu, M., Chantereau, J., Gerard, B., Ndjeunga, J., Bezançon, G. and Pham, J.L. (2006). Diversity of wild and cultivated pearl millet accessions (*Pennisetumglaucum [L.] R. Br*.) in Niger assessed by microsatellite markers. *Theoretical and Applied Genetics*, 114(1): 49-58.

Mutlu, S.S., Djapo, H., Ozmen, S.F., Selim, C. and Tuncel, N. (2015). Gamma-ray irradiation induces useful morphological variation in bermudagrass. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 43(2): 515-520.

Nei, M., Tajima, F. and Tateno, Y. (1983). Accuracy of estimated phylogenetic trees from molecular data. *Journal of Molecular Evolution*, 19: 153-170.

Negawo, A.T., Jorge, A., Hanson, J., Teshome, A., Muktar, M.S., Azevedo, A.L.S., Ledo, F.J., Machado, J.C. and S JONES, C.H.R.I.S. (2018). Molecular markers as a tool for germplasm acquisition to enhance the genetic diversity of a Napier grassm (*Cenchruspurpureus syn*. *Pennisetumpurpureum*) collection. *Tropical Grasslands - ForrajesTropicales,* 6(2).58-69.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular breeding*, *2*(3):225-238.

Skerman, P.J. and Riveros, F. (1990). Tropical grasses (No. 23). Food & Agriculture Organization (FAO). Rome, Italy. 832.