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Research Article

Exploitation of induced variation for enhancing the biomass production in rice (*Oryza sativa* L.)

S. Athira[1], Vinodhini Bhagya[1], G. Subashini[1], R. Muthuvijayaragavan[1], S. Rajeshwari[1], S. Manonmani[1], M. Raveendran[2], P. Jeyaprakash[1] and S. Robin[1]
[1]Centre for Plant Breeding and Genetics, TNAU, Coimbatore
[2]Department of Plant Biotechnology, Centre for Plant Molecular Biology, TNAU, Coimbatore
E-Mail: athiabhisobha@gmail.com

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Abstract

A study was undertaken for screening the EMS induced mutants of N22 for identifying high biomass mutants. In a population of 10000 mutants, 280 mutants were selected phenotypically and forwarded in which dry matter production along with 18 other traits including days to fifty per cent flowering, plant height, number of tillers per plant, number of productive tillers per plant, efficient tillering percentage, flag leaf length, flag leaf width, panicle length, number of filled grains per panicle, number of chaffy grains per panicle, spikelet fertility, hundred grain weight, grain length, grain breadth, wet biomass, grain yield , harvest index and vein density were characterized, Physiological traits namely SPAD reading, photosynthetic rate, transpiration rate, stomatal conductance and Ci/Ca ratio were also recorded. A total of 58 per cent had higher grain yield than N22 while a total of 214 mutants recorded with high dry matter production than N22. In terms of physiological efficiency, genotypes with high vein density are associated with high biomass as in the case of any C_4 plants. Wild type N22 has 50 minor veins and 8 major veins while 56 mutants possessed more than 50 minor veins and 10 major veins. Two mutants namely PL.No.36 and Pl.No.183 were found to be promising, which recorded high dry matter production with more number of minor veins, had high chlorophyll content and high photosynthetic rate.

Key words

Mutation, N22, Biomass, Vein density, photosynthetic rate, c_1/c_a ratio

Introduction

Rice (*Oryza sativa* L.), the oldest known domesticated grain, which is vital to the lives of billions of people around the globe. It serves as the central food for 2.5 billion people and it’s the single food crop, which covers about 9 percentage of the earth's arable land (Khush & Brar, 2002). Even though many attempts like hybridization, wide hybridization, heterosis breeding, and transgenic breeding approaches have been made to increase the rice yield potential, grain yield stagnation is one of the main issue to be addressed as it has been observed around the globe. To improve the yield potential a possible way is altering the morphology of the crop. There are two main elements which influence the grain yield, one of the factors is harvest index (HI) and the other is the biomass. As the harvest index (HI) is already high (ie, above 0.5), an alternative way for rice yield improvement is by increasing biomass because an additional improvement of the HI is nearly impossible. So here lays the importance of increasing biomass production.

The extent of genetic variations seen in a population often relates to its breeding system. As rice is self pollinated crop, variation which is essential for crop improvement is limited. Inducing mutations to alter several traits that found to be difficult to bring through cross breeding and other breeding procedures. Apart from using the genetic variation which is already present in the rice, techniques like mutation breeding is found to be a promising method to create novel variations The invention of mutation techniques has generated vast amount of genetic variability and played a significant role in rice breeding (Raina *et al.,* 2016). In rice, mainly physical and chemical mutagens have been used for inducing mutations Wu *et al.*, 2005).Ionizing radiations such as γ-rays can induce chromosomal aberrations. Chemical mutagens like ethyl methane sulphonate (EMS) acts mainly on base pairs of the DNA molecule and cause transitions. As chemical mutagens can cause a large number of desirable mutation, they are considered to be superior to physical mutagens (Nilan and Konzak, 1961). Majority of mutant varieties of rice were developed as direct mutants selected from mutated populations (Maluszynski,*et al* 2000).According to recent data base, 434 mutant varieties of rice have been released with improved characters.

In the present study, an attempt was made to screen high biomass Nagina 22 (N22) mutants with agronomic superiority using the EMS induced mutation breeding.

Material and Methods

With an objective to exploit the induced mutation for enhancing biomass in rice variety Nagina 22, this research work was conducted at Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore from 2017-2018 (plate 1). A large set of Ethyl methane sulphonate (EMS) (0.8%) induced mutants in the upland variety Nagina 22 were screened for biomass based on plant height, number of tillers per plant, wet biomass and dry matter production. The present investigation has been carried out by using mutants that were selected for high biomass.

A set of 10,000 mutants were evaluated previously and based on phenotypic selection, 280 plants were found to have high biomass. All these 280 mutants were now observed for different traits in this study. In order to exploit high biomass mutants, dry matter production along with 18 other traits including days to fifty per cent flowering, plant height, number of tillers per plant, number of productive tillers per plant, efficient tillering percentage, flag leaf length, flag leaf width, panicle length, number of filled grains per panicle, number of chaffy grains per panicle, spikelet fertility, hundred grain weight, grain length, grain breadth, wet biomass, grain yield, harvest index and vein density (number of major veins and number of major veins) were observed. For taking vein density, fresh third leaf was collected from selected mutants and was hand cut from widest part of the leaf using a razor blade. The section was mounted on a microscope slide, and a small drop of water is smeared on it. Sections were viewed using a light microscope and images captured using a camera. The number of major and minor veins were counted and recorded separately.

Besides the morphological traits, physiological traits namely SPAD reading, photosynthetic rate, transpiration rate, stomatal conductance and Ci/Ca ratio were recorded. SPAD reading was taken using Minolta SPAD (Soil and Plant Analysis Division) chlorophyll meter. Ci/Ca ratio was taken for assessing photosynthetic efficiency for greater biomass and yield production. All other physiological characters were recorded using Infrared gas analyzer is a portable photosynthetic system (LICOR – Made- LI 6400 version 5).

Results and Discussion

All the 280 mutant plants selected were observed individually. Flowering pattern in the mutants differs from N22, in a way that all are late than N22. Late maturing varieties found to have more biomass production than early varieties. In late varieties leaf senescence will be delayed so that the leaves will remain photosynthetically active for long time, resulting in significantly larger dry-matter production as reported by Kato *et al.*(2004). However in the case of plant height, out of 280 mutants, 117 were observed to be taller than N22. Number of tillers of 227 mutants found to be higher than that of wild type N22.The biomass yield was positively influenced by the number of tillers (Baligar and Fageria, 2007). A total of 242 plants recorded higher number of productive tillers than that of N22. A set of 69 percentage of total mutants recorded high efficient tillering percentage than N22 (Table 1).

Photosynthate assimilation rates are more related to flag leaf traits, which in turn assessed for its length and breadth. The new plant type concept of rice shows that improved flag leaf architecture will increase the rate of photosynthetic rates in turn enhances the biomass production proved by Bing, *et al*(2006). In this study 74 percentage and 57 percentage of the mutants found to have more flag leaf length and breadth than the wild type respectively (Table 2). The sink capacity of panicles influences dry-matter production which in turn determines the biomass yield (Kato et al., 2004). A total of 161 mutants exhibited long panicle than N22, and added an advantage to this study.

Spikelet fertility found to have a positive association with total biomass production (Ndour *et al.*, 2016). Spikelet fertility percentage of the mutants exhibited a range from 60 to 93 percentage. Out of 280 mutants, 31 had high fertility percentage than the wild type N22.Hundred grain weight showed negative association with biomass production, reported by Venkanna, *et al* (2014). In case of hundred grain weight 54.65 percentage of the mutants showed low value than the N22.

Even though a total of 180 mutants recorded high wet biomass than N22, selection for wet biomass was not reliable. Grain yield is one of the important characters associated with biomass production and out of the 280 mutants 58 percentage of total mutants exhibited high grain yield than N22. Wu *et al.*(2008) reported that dry matter production found to be the prime character determining the biomass yield in rice and relatively more emphasis was given to dry matter production.

Leaf venation architecture found to have high association with vein density, i.e. the number of major and minor veins. The vein density has an important role in enhancing biomass and yield (Scarpella, *et al* 2003).Identification of such high vein density candidates, producing dense panicles, will be a major contribution towards the improvement of photosynthesis in rice, thereby increasing the biomass yield (Nawarathna *et al.*, 2017). The mutants showed altered patterns for vein density. High biomass mutants showed more number of major and minor veins. Wild type N22 has 50 minor veins and 8 major veins. Some of the high biomass mutants possessed 61 minor veins and 10 major veins. Mutants with high vein density from the present study can be exploited for the understanding the causes of genetic regulation leading to the production of C_4 rice. SPAD reading (which shows the chlorophyll content of leaf) found to be high for all high biomass mutants (Table 1). This may be due to the increase in rate of photosynthesis rates in accordance with Hidayati *et al*.(2016).

Induction of mutation using EMS was found to be successful in this study. As expected, this programme resulted in creation of variation and there by broadening of genetic base. Because it is observed from the results that, some of the mutants performed better than the wild type N22. Out of the total 280 plants 41 percentage of mutants performed better than N22 for plant height, 81 percentage for number of tillers, 86 percentage for number of productive tillers, 69 percentage for efficient tillering percentage, 74 percentage for flag leaf width, 57 percentage for panicle length, 45 percentage spikelet fertility, 64 percentage for hundred grain weight, 58 percentage for grain yield and 76 percentage for dry matter production.

Dry matter production showed significant and positive correlation with days to fifty percent flowering, plant height, number of tillers, number of productive tillers, panicle length, flag leaf length, wet biomass, SPAD reading and grain yield which are in accordance with the findings of Abarshahr *et al* (2011) and Haque *et al* (2015).

Based on the association studies, dry matter production is found to be correlated with days to fifty percent flowering, plant height, number of tillers, number of productive tillers, flag leaf length, panicle length, number of major and minor veins, SPAD reading. Totally 25 plants with high dry matter production (more than 123 g per plant *i.e*. 250% dry matter more than the wild type) were specifically selected (Table 3)

All the selected mutants were late flowering (52 to 70 days) than the wild type parent N22(45 days). Kato *et al.*, (2004) reported that biomass accumulation was found to be increased with increase in duration of the crop. Plant height of all mutants recorded above 100 cm. except four mutants namely, Pl.No.193, Pl.No.71, Pl.No.170 and Pl.No.49. Number of tillers per plant and number of productive tillers per plant was highest in Pl.No.70. All mutants recorded with long panicle than the N22. Lengthier panicle was positively associated with biomass production (Kato, 2004).

In this study the high biomass mutants also recorded with high number of minor veins when compared to the wild type N22. Five mutants namely, Pl.No.36, 183, 161, 257 and 194 were found to have specifically high number of minor veins coupled with high dry matter production. Such mutants may be utilized for further improvement in rice breeding programme for selection of lines with more photosynthetic efficiency as supported by Nawarathna *et al.*, (2017). The mutagenic effect on N22 displayed a series of mutants with more number of major veins (10 veins) that were different from the wild type, which recorded 8 major veins. No significant variation was found among the mutants for the trait number of major veins. Even though the wet biomass of the mutants Pl.No.149, 152, 148, 160, 49, 218 and 223 were found higher, the corresponding grain yield was lower which confirms the wet biomass is not reliable to be selected for sorting out high biomass mutants. The trait wet biomass is more influenced by the field conditions (moisture percentage at the time of harvesting). Two mutants namely PL.No.36 and Pl.No.183 were found to be more promising among all the selected 25 mutants, which recorded high dry matter production with more number of minor veins, which is the reliable or dependable genetic make up for high biomass.

Further the selection of mutants Pl.No.36 and Pl.No.183 is attributed to the added reasons of high chlorophyll content (above 40) when compared to wild type N22 (25) and also had high photosynthetic rate (37.65mmol/cm^2/s for Pl.No.183 and 40.47 mmol/cm^2/sfor Pl.No.36). Besides, these two mutants recorded with low rate of transpiration (below 8mmol/cm^2/s) than the N22 (9.62 mmol/cm^2/s) and a fairly high stomatal conductance (above 0.5 mol $H_2O/m^2/s$) and Ci/Ca ratio (above 0.7) when compared with N22 (0.46

mol $H_2O/m^2/s$ and Ci/Ca ratio of 0.62). Leaf photosynthetic capacity was associated with its higher stomatal conductance (Adachi *et al.*, 2011).The increase in photosynthetic rate, stomatal conductance, Ci/Ca ratio and decrease in transpiration rate may be due to the increased number of veins in that mutants. Increase in number of veins and the change in arrangement of mesophyll resulted in an enhancement in photosynthetic efficiency thereby improved the biomass accumulation in rice (Smillie, *et al* 2012).

These two mutants namely Pl.No.36 and Pl.No.183 (plate 2and 3) adjudged as the top rankers among all the selected mutants and they can be exploited for further biomass improvement programmes. They can be crossed with wild type N22 for developing mutmap populations. Such programmes are required to understand the function of individual gene, and their interactions among themselves as well as with the environment. Mutants produced by induced mutation facilitate unveiling the causal relationship between coding or regulatory sequences and plant performance. Induced mutations can be efficiently integrated with genomics, transcriptomics, proteomics and metabolomics studies to understand the phenome. However, limited information is available on their phenotypic evaluation and only small subset of this mutant is freely available for unrestricted use (Mohapatra *et al*., 2014).

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Table 1. Mutants selected for high biomass (M_4) related traits

Pl.No	DMP	DFF	PH	NT	NPT	FLL	PnL	WB	GY	Major	Minor
36	138.00	55	115.0	62	51	42.0	21.0	195.00	54.00	10	58
33	136.00	52	111.0	36	34	31.0	20.0	168.00	44.00	9	52
183	136.00	69	116.5	60	52	36.0	20.0	192.00	56.00	10	59
193	135.00	56	98.5	65	41	35.0	21.0	160.00	50.00	9	52
269	132.50	69	114.5	35	30	30.0	19.5	164.00	32.00	8	51
152	132.00	70	112.0	55	45	26.0	21.0	219.50	42.00	9	51
248	130.00	69	100.0	48	35	32.0	19.5	159.50	39.00	8	49
71	129.00	67	96.5	49	47	26.0	20.0	148.00	30.50	8	51
160	129.00	69	105.0	42	36	29.0	22.0	234.00	40.00	9	52
148	128.00	70	115.0	65	54	35.0	24.0	240.00	35.00	9	61
170	128.00	69	94.0	52	32	30.0	20.0	152.50	32.00	10	49
200	128.00	68	100.0	42	34	33.0	20.5	140.00	31.50	9	51
202	128.00	68	104.0	35	32	30.0	23.5	145.00	28.00	8	51
49	126.50	65	94.0	60	50	32.0	19.5	221.00	36.00	9	56
166	126.20	67	110.0	52	36	29.0	22.0	166.00	32.00	9	52
38	126.00	64	100.0	37	35	30.0	19.0	140.00	25.00	8	48
161	126.00	68	100.5	47	34	30.0	20.0	159.00	24.00	9	55
149	125.50	69	102.0	60	48	27.5	21.0	202.50	26.00	8	54
70	125.40	65	105.0	36	32	28.0	23.0	158.00	38.00	8	51
257	125.00	69	110.0	48	46	31.0	21.0	215.00	27.00	9	55
194	124.70	68	118.0	50	47	29.0	25.0	184.00	28.00	8	55
18	124.50	65	120.5	52	42	39.0	19.5	202.00	27.00	8	52
232	124.00	69	102.0	34	32	32.5	20.0	165.00	30.00	9	51
218	123.50	69	102.0	48	38	28.5	19.0	200.00	28.00	8	47
223	123.40	70	102.0	56	31	29.5	19.5	206.00	25.00	8	52
Max	**138.00**	**70.0**	**120.5**	**65**	**54**	**42.0**	**25.0**	**240.00**	**56.00**	**10.0**	**61.0**
Min	**123.40**	**52.0**	**94.0**	**34**	**30**	**26.0**	**19.0**	**140.00**	**24.00**	**8.0**	**47.0**
N22	**48.88**	**45.5**	**97.6**	**25**	**17**	**31.1**	**18.7**	**82.23**	**22.38**	**8.6**	**50.9**

Table 2. Physiological traits for the selected mutants (M_4)

Pl. No.	SPAD	PR	TR	SC	Ci/Ca
36	43.4	40.47	7.52	0.57	0.78
33	38.5	38.75	8.6	0.52	0.88
183	39.5	37.65	7.1	0.72	0.89
193	37.9	37.52	7.71	0.55	0.85
269	36.8	37.46	8.7	0.38	0.76
152	41	37.26	8.91	0.71	0.75
248	39.1	36.35	9.94	0.61	0.75
71	34	35.2	8.57	0.48	0.78
160	39.5	33.72	10.06	0.68	0.81
148	41.2	37.19	8.86	0.5	0.76
170	39.1	31.97	11.89	0.77	0.8
200	34.9	28.49	9.26	0.52	0.77
202	37.5	28.41	13.79	0.5	0.8
49	40.6	36.33	9.5	0.56	0.79
166	39.1	27.21	10.39	0.71	0.82
38	34.6	26.74	8.5	0.57	0.8
161	38.7	34.18	9.5	0.45	0.79
149	42.1	38.11	8.5	0.5	0.8
70	34	32.73	9.75	0.59	0.75
257	39.7	38.59	7.65	0.42	0.68
194	40.1	36.65	6.5	0.61	0.69
18	35.7	28.36	8.5	0.72	0.72
232	39.2	34.72	10.2	0.77	0.74
218	34.4	26.5	10.66	0.56	0.69
223	41.5	35.4	11.2	0.55	0.69
Min	**34**	**26.5**	**6.5**	**0.38**	**0.68**
Max	**43.4**	**40.47**	**13.79**	**0.77**	**0.89**
N22	**24.95**	**24.95**	**9.62**	**0.46**	**0.62**

Table 3.Correlation studies for morphological and physiological traits in mutants (M_4)

	DFF	PH	NT	NPT	FLL	FLW	PnL	HGW	GL	GB	Major	Minor	SPAD	WB	GY	HI	DMP
DFF	1	0.253**	0.418**	0.377**	0.056	0.011	0.206**	0.035	0.107	-0.009	0.283**	0.318**	0.541**	0.749**	0.375**	-0.639**	0.790**
PH		1	0.127*	0.281**	0.429**	0.294**	0.253**	-0.123*	-0.021	0.068	0.227**	0.249**	-0.026	0.469**	0.318**	-0.196**	0.417**
NT			1	0.681**	0.080	0.162**	0.052	0.152*	0.043	0.011	0.177**	0.271**	0.447**	0.479**	0.321**	-0.191**	0.410**
NPT				1	0.089	0.100	0.111	0.064	0.029	0.084	0.241**	0.354**	0.310**	0.526**	0.284**	-0.205**	0.411**
FLL					1	0.398**	0.101	-0.061	-0.062	-0.042	0.106	0.097	-0.103	0.190**	0.133*	-0.072	0.175**
FLW						1	0.114	-0.108	0.011	0.061	0.120*	0.158**	-0.067	0.101	-0.006	-0.025	0.054
PnL							1	-0.007	0.150*	-0.053	0.096	0.081	0.148*	0.175**	0.096	-0.191**	0.191**
HGW								1	0.090	0.000	0.005	0.018	0.194**	-0.017	-0.013	0.001	-0.008
GL									1	0.085	-0.036	-0.068	0.101	0.052	0.025	-0.046	0.050
GB										1	0.135*	0.057	-0.043	0.023	-0.067	-0.016	-0.010
Major											1	0.511**	0.161**	0.444**	0.405**	-0.139*	0.415**
Minor												1	0.268**	0.460**	0.364**	-0.091	0.392**
SPAD													1	0.535**	0.381**	-0.365**	0.545**
WB														1	0.643**	-0.546**	0.912**
GY															1	0.059	0.706**
HI																1	-0.624**
DMP																	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed)

DFF- Days to fifty percent flowering (days), PH – Plant height (cm) , NT – No. of tillers, NPT – No. of productive tillers, FLL – Flag leaf length (cm), FLW – Flag leaf width (cm), PnL- Panicle length (cm), , HGW- Hundred grain weight (g), GL – Grain length (mm), GB- Grain breadth (mm), Major- No. of major veins, Minor- No. of minor veins, WB-Wet biomass (g), GY- Grain yield (g),SPAD – SPAD reading, HI – harvest index,DMP – Dry matter production (g)

Fig. 1. Field view of N22 Mutants

Fig. 2. High biomass mutant (Pl.No.36)and N22

Fig. 3. High biomass mutant (Pl.No.183) and N22