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REGULAR ARTICLE

Nitrous acid-induced variations in some quantitative traits of foxtail millet (*Setaria italica* (L.) P. Beauv.)

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Abstract

The effects of nitrous acid on the agronomic parameters of foxtail millet were investigated. Seeds with nitrous acid treatments and untreated seeds (control) were planted in a Randomized Completely Block Design (RCBD) for two generations. The following data collected: percent germination, plant height, leaf number, length, and width, fresh and dry weights, number of days to 50% flowering, panicle length, and weight, and 1000-seed weight. Significant variations were observed in plant height, leaf number and length, fresh and dry weights, panicle length and weight, and 1000-seed weight in the control. Plant height, leaf length, fresh weight and dry weight had highest mean values at 0.1% nitrous acid. Number of leaves and panicle length and weight were highest at 0.4% concentration, while 1000-seed weight was highest with 0.3% nitrous acid. From this experiment, nitrous acid proved to be effective in inducing mutations that increased quantitative traits of the foxtail millet. The mutations have great potentials which can be harnessed for use in a breeding program.

Key words: Breeding; mutation; traits; variation; chemical mutagen

Introduction

Foxtail millet (*Setaria italica*), is one among the important grass family Poaceae, which is mainly cultivated in low water requirement and can be planted as second season crop due to its short life-cycle. Foxtail millet has high contents of protein and other nutrient potentials when compared to staple cereals (Thathola et al., 2011). It is a self-pollinating plant with miniature sized floral organs which traditional hybridization for new cultivar appears unfeasible (Siles et al., 2004). Crop improvement programs mainly focus on farmer's demands and selection of desirable morphological characters (Aliyu et al., 2016).

In crops with limited variability, it is particularly important to utilize available natural or induced genetic diversity and mutagenesis (Martin et al., 2009). Nitrous acid has been shown induce in vivo DNA mutation, giving rise to A-T-->G-C transition (Sidorkina et al., 1997). The amino acid can therefore be modified to change the phenotype. As most of the mutations are recessive, the plants for selection must be self-fertilized and advanced to at least the M_2 after mutagenic treatment. The aim of this study was to determine the effects of nitrous acid on foxtail millet and the optimal concentration that results in an increase in the agronomic traits studied.

Materials and methods

Study area

The study was conducted in the botanical garden of the Department of Biological Sciences, Ahmadu Bello University, Samaru, Zaria, (Lat 11° 11' N; Long 7°E 38', Altitude

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660m above sea level). Samaru lies in the northern guinea savanna agro ecological zone of Nigeria with a mean annual rainfall of about 1011±16.1 mm (CV=16%) from 1960 to 2003 (Oluwasemire and Alabi, 2004). Zaria has three distinct seasons; namely the hot dry season from March to May, the warm rainy season from June to September, and the cool dry season from November to February. The area has an average temperature of 27°C with minimum and maximum temperatures of 15.6 and 38.5°C respectively (NCAT, 2008).

Source and treatment of materials

Seeds (moisture content 10-12%) of foxtail millet were obtained from The Institute for Agricultural Research/ Faculty of Agriculture, Ahmadu Bello University, Zaria, Kaduna State. To raise the first mutant (M_1) generation, seeds were first soaked in distilled water for 4 hours. Freshly prepared aqueous dilutions of nitrous acid (0.00%, 0.1%, 0.2%, 0.3% and 0.4%) were then used to treat the seeds for 4 hours. Treated seeds were then thoroughly washed in running tap water to remove any residual mutagens (According to Bind et al., 2016).

Experimental design

Treated and control seeds were sown in a Randomized Completely Block Design (RCBD) with 40×75cm intra and inter space. Each treatment was replicated four times.

To raise the M_2 generation, randomly selected healthy seeds harvested from M_1 generation of each treatment of fast neutron, were planted in Randomized Complete Block Design (RCBD) with four replications in the botanical garden in 40×75cm intra and inter space.

Data collection

The parameters measured were: Seed germination percentage: Twenty seeds from each of the treatments were placed on Whatman filter paper in petridish (9cm X 2cm). Each petridish was moistened with 2ml/ plate of distilled water and kept cupboard room temperature. The germination percentage was counted on the 7th day. Plant height (cm), leaf length (cm) and length of panicle (cm) were determined using a meter rule. leaf width (cm), fresh weight (g), dry weight (g), weight of panicle(g) and seed weight for 1000 seeds (g) were using a weighing balance. number of leaves and days to 50% flowering determined by counting.

Data analysis

One-way ANOVA was used to determine any significant differences between the means of different treatment concentrations. DMRT was used to separate the means at $P<0.05$ using the SAS software V. 2002 for Windows.

Results and discussion

Table 1 shows the performance foxtail millet exposed to different concentrations of nitrous acid in the M_1 generation. There were significant variations in plant height, number of leaves, fresh weight, dry weight, days to fifty percent flowering, panicle length, panicle weight ($P<0.05$). The variation in the traits across treatments may be due to deamination of nitrogenous bases of DNA which alters forms of a triplet sequence (Uno et al., 2001). Variation in some agronomic parameters was also reported in Fonio millet (Acca: Hausa) treated with nitrous acid (Animasaun et al., 2014).

Plant height increased significantly at 0.1 concentration. This also agrees with the findings of Eswari et al. (2014) who reported increase in plant height of finger millet due to EMS mutagenesis. Number of leaves, Fresh weight and dry weight increased with increase in concentration of the mutagen and was maximum at 0.4%. *Vicia faba* treated for 24 hours with nitrous acid (AL-Shamma and Sahib, 2014). This may be due to activation of increase in production of growth hormones. The significant decrease in days to 50% flowering may be as a result of nitrous acid deamination of DNA resulting mutation which caused variability in various characters. Animasaun et al. (2014) reported similar findings in fonio millet. Panicle length and weight showed a similar trend of increase with increase in concentration of nitrous acid where 0.1% maximum value for panicle length and weight while 0.3% maximum value. Similar increase in yield characters was reported in soya beans (Mensah et al., 2016).

Table 2 shows the mean performance of foxtail millet exposed to different concentrations of nitrous acid at M_2 generation. Significant variation was observed in plant height, number of leaves, leaf length, fresh and dry weights, panicle length and weight and 1000-seed weight. The M_2 showed more variation than M_1. This finding is similar to reports on peanut (Wang et al., 2015). This is to be expected at M_2 (mutation that affected meiosis) while the variation observed in the M_1

generation is not phenotypic but a result of heritable characters produced by the effect of fast neutrons on somatic cells. This may also probably be because most mutations are recessive; therefore, variations can only be expressed in the M_2 generation after segregation has occurred during meiosis in the M_1 generation. Plant height, leaf length, and fresh and dry weights were highest at 0.1% nitrous acid. This could be due to promotion of physiological and biological processes necessary for seed germination which includes enzyme activity induced by nitrous acid.

Number of leaves, and panicle length and weight were highest at 0.4% while 1000-seed weight was highest at 0.3% nitrous acid (Table 2). These changes may be due to random mutagenic effects of nitrous acid occurring and varying at different concentrations. Similar dose-dependent variations in quantitative traits was reported in finger millet (Muduli and Misra, 2008), in cowpea (Girija et al., 2013), foxtail millet (Esson et al., 2016) and Isabgol (*Plantago ovata* Forsk.) treated with EMS (Mishra and Khan, 2014).

Conclusions

Nitrous acid induces variation in plant height, number of leaves, leaf length, fresh and dry weights, panicle length and weight, and 1000-seed weight of the Foxtail Millet. In the M_1 generation, these parameters increase; in the M_2 there are more variations in plant height, number of leaves, leaf length, fresh and dry weight, panicle length and weight, and 1000-seed weight. 0.1% concentration gives better performance in traits associated with growth (plant height, number of leaves, leaf length,

fresh weight, dry weight) while 0.4% and 0.3% produce better performance for traits associated with yield (panicle length, panicle weight, 1000-seed weight).

Table 1. Mean Performance of Effects of Different Concentrations of Nitrous Acid on agronomic traits of Foxtail Millet at M_1 generation

NA	PGERM	PHT (cm)	NL	LL (cm)	LW (cm)	FW (g)	DW (g)	DPFL (days)	PL (cm)	PW (g)	TSW (g)
Control	83.75^{a}	34.18^{b}	5.70^{b}	14.43^{a}	1.33^{a}	2.10^{b}	0.78^{b}	42.75^{a}	4.83^{c}	0.75^{b}	2.601^{a}
0.10%	87.50^{a}	37.51^{a}	5.88^{b}	15.76^{a}	1.42^{a}	3.08^{a}	1.35^{a}	34.35^{b}	6.08^{a}	1.08^{a}	2.60^{a}
0.20%	86.25^{a}	34.42^{b}	5.00^{b}	15.59^{a}	1.40^{a}	2.98^{a}	1.43^{a}	34.50^{b}	5.13^{bc}	1.10^{a}	2.75^{a}
0.30%	82.50^{a}	35.10^{b}	5.75^{b}	15.57^{a}	1.47^{a}	3.13^{a}	1.45^{a}	35.75^{b}	5.69^{ab}	1.13^{a}	2.78^{a}
0.40%	88.75^{a}	35.91^{ab}	6.75^{a}	16.15^{a}	1.48^{a}	3.43^{a}	1.4^{a}	41.75^{a}	5.99^{a}	1.03^{a}	2.94^{a}
SE	3.17	0.59	0.36	0.76	0.05	0.27	0.15	1.21	0.22	0.08	0.15

NOTE: Means with the Same Letter within a Column are not Significantly Different at P≥0.05
PGERM- Percentage Germination, PHT- Plant Height at Maturity, NL- Number of Leaves, LL- Leaf Length LW-Leaf Width, FW- Fresh Weight, DW-Dry Weight, DPFL- Days to 50% Flowering, PL- Panicle Length PW- Panicle Weight, TWS- One Thousand Seed Weigh, SE- Standard error, M_1-Second mutant generation, NA- Nitrous Acid

Table 2. Mean Performance of Effects of Different Concentrations of Nitrous Acid on agronomic traits of Foxtail Millet at M_2 generation.

NA (%)	PGERM (%)	PHT (cm)	NL	LL (cm)	LW (cm)	FW (g)	DW (g)	DPFL (days)	PL (cm)	PW (g)	TSW (g)
0.00	81.25^{a}	25.10^{b}	5.5^{bc}	9.75^{b}	1.28^{a}	1.30^{b}	0.45^{b}	57.25^{a}	3.68^{c}	0.86^{ab}	2.84^{c}
0.10	78.75^{a}	31.95^{a}	6.00^{ab}	15.50^{a}	1.43^{a}	2.05^{a}	1.10^{a}	55.75^{a}	4.75^{a}	0.75^{ab}	3.19^{b}
0.20	83.75^{a}	29.73^{a}	5.63^{bc}	13.04^{ab}	1.37^{a}	1.33^{b}	0.58^{b}	52.25^{a}	4.63^{ab}	0.91^{ab}	3.23^{b}
0.30	85.5^{a}	29.76^{a}	5.00^{c}	13.26^{ab}	1.29^{a}	1.33^{b}	0.68^{ab}	54.25^{a}	4.18^{bc}	0.69^{b}	3.65^{a}
0.40	82.5^{a}	31.41^{a}	6.63^{a}	14.67^{ab}	1.48^{a}	1.35^{b}	0.73^{ab}	50.25^{a}	5.05^{a}	1.00^{a}	3.21^{b}
SE±	3.66	1.22	0.39	1.5	0.06	0.2	0.16	2.08	0.17	0.09	0.11

NOTE: Means with the Same Letter within a Column are not Significantly Different at P≥0.05
PGERM- Percentage Germination, PHT- Plant Height at Maturity, NL- Number of Leaves, LL- Leaf Length LW-Leaf Width, FW- Fresh Weight, DW-Dry Weight, DPFL- Days to 50% Flowering, PL- Panicle Length PW- Panicle Weight, TWS- One Thousand Seed Weigh, SE- Standard error, M_2-Second mutant generation, NA- Nitrous Acid

Authors contribution

This work was initiated, designed and carried out by Esson, E. A. and Adebola, M. I. as part of Esson E.A. Master of Science research thesis and was supervised by Adamu, A.K. and Adelanwa, M. A.

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