

EFFECTS OF NITROUS ACID ON GROWTH AND YIELD OF *DIGITARIA EXILIS* (HALLER)

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ABSTRACT

Digitaria exilis seeds have several economic uses with long shelf life and impressive nutritional qualities. The crop is faced with problems of low yield and small-sized grains among others, thus, the crop is neglected and little information is available on its improvement by mutagenesis. This work assessed effect of Nitrous acid on growth and yield of *Digitaria exilis*. *D. exilis* seeds were exposed to 0.1M Nitrous Acid for varying time of 2 hr, 4 hr, 6 hr and 8 hr, untreated seeds were used as control. The seeds were sown after treatment with Nitrous acid and effect on the quantitative and qualitative characters studied. Germination and seedling survival decreased with increase in time of exposure. Plant height, number of leaves, tiller/plant, and leaf length were significantly reduced at 6 and 8 hrs of treatment. All treatments induced early maturity but with low seed set and yield among 6 and 8 hr plants. Generally, the performance of the treated plants was better with short exposure time and 4 hr of treatment produced best agronomical traits and improved yield. Erect plants were obtained with 2 and 4 hr treatment which is a promising trait for mechanical harvesting of the crop. This study concluded that 4hr of exposure of *D. exilis* to 0.1M Nitrous acid could induce useful variability for improvement of the crop in a breeding programme.

KEYWORDS: *Digitaria exilis*, early maturity, growth and yield, mutation breeding, nitrous acid, seedling survival

INTRODUCTION

Among the dominant cultivated grasses (family; Poaceae) of the tropical and sub-tropical regions is *Digitaria*, which consists of about 300 species of both annual and perennial grasses (Bodgan, 1977). Several species of *Digitaria* have been identified, of which about 34 of them are indigenous grasses and a few of them are suspected to have been introduced to West Africa recently (Kativu and Mhlanga, 1995).

D. exilis is the most important member of a diverse group of wild and domesticated *Digitaria* species native to the savannas of West Africa where it is fondly called *acha*, *fonio*, *fundi* or *hungry rice*. The crop is a minor cereal which is cultivated throughout West Africa from Senegal to Lake Chad, Nigeria and even in the Dominican Republic (Morales-Payan *et al.*, 2003). *Digitaria* is a small-seeded cereal that has great genetic developmental potential. The long shelf life and keeping qualities of the grains are particularly important in its potential for replacing the super cereal crops in industrial processes for food production of tomorrow. Among the Hausas in Nigeria, Benin, Togo and Ghana, *acha* is made into a special conspicuous food type called '*wusu-wusu*'. The flour is made into thick, unfermented porridge (tuwo-acha), and fermented grains are used for thin porridge (kunu-acha). Several other cultural, economic and nutritional utilization of the crop have been reported (Kuta *et al.*, 2003; Morales-Payan *et al.*, 2003).

Although Acha is important for its organoleptic qualities, its production is faced with a number of

problems out of which grain yield is the most significant. Grain yield of the crop varies from 600 – 800kg/Ha and could reduce to between 150 – 200 Kg/ha on a very poor soil (Maji *et al.*, 2003). The small-sized grain are fugacious, shattered and matures unevenly and therefore, the crop has remained underexploited and generally unimproved, thus making it unappealing to farmers for commercial production (Maji *et al.*, 2005; Morakinyo and Awojobi, 1991).

In recent time, chemical mutagens have become important tools in crop improvements. These mutagens are used to induce variability and extended gene pool from which crops disease resistance, abiotic resistance, high yield and fortified nutrients are produced (Chowdhury and Tah, 2011). Effect of mutagens on crops such as maize, rice, sesame and sunflower had been reported (Meshenkov, 1986; Ricardo and Ando, 1998; Mensah *et al.*, 2007; Skoric *et al.*, 2008).

In view of the fact that very little information is available on the improvement of *Digitaria exilis* by mutagenesis, this work aimed at examining the effect of different doses of Nitrous oxide on the growth characteristics of the crop to create variability on which selection could be based for the purpose of breeding and improvement.

MATERIALS AND METHODS

D. exilis seeds used for this study were collected from the National Cereal Research Institute, Moor Plantation, Ibadan, Oyo State, Nigeria. Seed colour, shape, seed

coat texture and weight of 100-grains were recorded before and after the treatments were applied. Dry seeds of 10 g each were weighed using Mettler balance (Model RA 50- REPTech) into four separate petri-dishes and soaked in with freshly prepared Nitrous acid of 0.1M for varying times of 2hr, 4hr, 6hr and 8hr. After the treatment, seeds were removed from the acidic solution and washed under running tap to remove residual acid, then air-dried and packed in labeled seed envelopes in preparation for sowing. The control seeds were soaked in distilled water for 3 hr and then air-dried prior to sowing

Seeds were sown in nursery beds (0.5 m x 0.5 m) rich in loamy soil prepared for each of the treatment time and the control in the net house facility at Biological Garden, University of Ilorin, (N 08° 28' 53.3", E 04° 40' 28.9"), Ilorin, Nigeria. On the third week after sowing, three (3) plants were transplanted into a five liter capacity planting bag filled with soil and labeled according to the seedlings treatments. Five (5) replicates were made for each of the treatment and control in a randomized complete block design. Watering and hand weeding were done as at when due.

Data were taken on the following parameters; days to emergence, percentage germination, plant height, number of leaves per plant/tiller, number of nodes per plant/tiller, inter-node length, leaf width, leaf length, number of tiller/plant, and leaf sheath length. The yield characters studied were days to maturity, days to 50% flowering, peduncle length, spike length, number of spike /plant/tiller number of spikelets per/plant, percentage seed set and 100-grain weight. Vegetative characters were evaluated 3 weeks after transplanting and at maturity.

Days to maturity was taken as the difference between the date of sowing and the date of inflorescence emergence from the sheath of the flag leaf. The weight of 100 seeds was determined using Mettler balance (Model RA 50- REPTech). Five different measurements were taken in all cases and the means were calculated. Plant colour was observed at 3 weeks after emergence, while growth pattern and pubescence were observed at maturity.

Data obtained were subjected to analysis of variance (ANOVA) using SPSS Microsoft Window Operating System Software version 16.0. The means obtained were separated with Duncan's Multiple Range Test at the probability level of $P \leq 0.05$.

RESULTS

Seed colour, shape and texture were the same before and after treatment. Germination commenced on the 4th day after sowing. Mean days to emergence for the control seeds was 6.2 with percentage germination of 90.3%. The mean days to emergence for the nitrous acid treated seeds ranged between 4.8-7.8 days (Fig 1a). The 2 hr treated seeds emerged 4.8 days after sowing but with lower percentage germination (78.18%) while 4 hr treated-seeds emerged 6.2 days after sowing with the highest percentage germination (81.12%). Six (6) hr and 8 hr treated seeds had longer days to germination and very low percentage seed germination as shown in Fig 1a. Figure 1b showed the percentage survival of the seedlings after transplant which decreased with increase in treatment time. While 83.3 and 80.4% of 2 hr and 4 hr treated seedlings survived respectively, about 40% of 6 hr seedlings and 44% of 8 hr seedlings died few days after transplanting. However, survival percentage was 94.3% for control seedlings (Fig 1b). Quantitative characters considered 3 weeks after sowing showed that the leaf breadth, plant height and leaf length were highest in the 4 hr treated plants. Higher treatment exposure times of 6 and 8 hr induced less vegetative growth as presented in Table 1.

The vegetative and growth characters considered at maturity revealed significant difference in plant height, leaf length, number of tiller/plant, number of leaf /tiller, number of nodes/tiller and internode length (Table 2). For these characters, the 4 hr treated plants consistently maintained the lead while the least values were recorded among 8 hr plants in most cases. However, significant early maturity was observed in chemically treated plants where all treatment exposure time achieved maturity earlier than the control (Table 2).

The fruit and yield characteristic of *D. exilis* in response to treatments are presented in Table 3. Fruit setting and weight of 100 grains decreased as the treatment period increased. In comparison to the control plants, 4 hr plants had optimum yield and least yield was recorded among 8 hr plants. Meanwhile, differences were observed in stem base and leaf pigmentation among the treated plants (Table 4). The 2 hr and 4 hr nitrous acid-treated plants had reddish stem base and reddish-green leaf sheaths with pale green leaf, while 6 hr and 8 hr plants had reddish stem base, reddish leaf sheaths and green leaves. The summary for qualitative characters such as the growth pattern, leaf and stem pigmentation as well as pubescence is shown in Table 4.

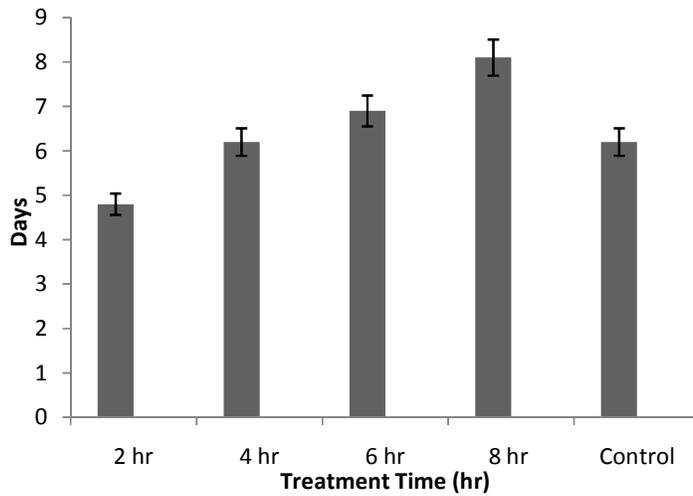


Fig 1a: Mean number of days to germination of *D. exilis* treated with nitrous acid

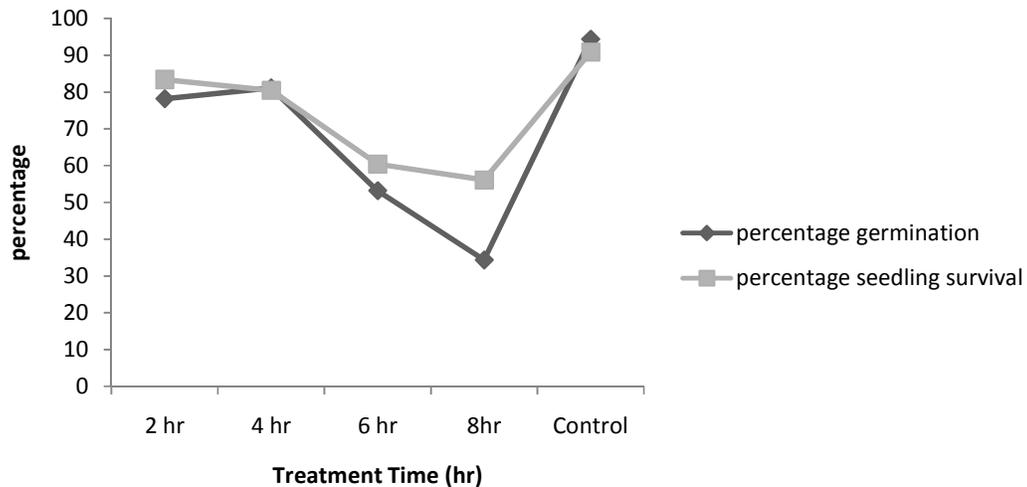


Fig 1b: Percentage germination and percentage seedling survival of nitrous acid treated *D. exilis*

Table 1: Quantitative characteristics of nitrous acid treated *D. exilis* at 3weeks after transplanting

Treatments	No of leaves	Leaf length (cm)	Leaf breath (cm)	Plant height (cm)
2 hr	3.2 ^a ±1.30	1.5 ^c ±0.40	0.3 ^a ±0.01	3.14 ^b ±0.4
4 hr	2.6 ^b ± 0.92	2.9 ^a ±0.61	0.4 ^a ±0.02	3.92 ^a ±0.33
6 hr	2.6 ^b ±0.94	2.4 ^b ±0.83	0.3 ^a ±0.01	2.90 ^c ±0.52
8 hr	2.1 ^c ±0.94	1.2 ^c ±0.71	0.3 ^a ±0.04	2.60 ^c ±0.80
Control	3.1 ^a ±1.11	2.8 ^a ±0.90	0.4 ^a ±0.00	3.82 ^a ±0.64

Values with same alphabet are not significantly different ($P < 0.05$) along the column.

Table 2: Quantitative characters (at maturity) of nitrous acid treated *D. exilis*

TRT	PH (cm)	NTP	NLT	LL (cm)	NPT	IL (cm)	LSL (cm)	LB (cm)	NDM
2 hr	85.40±4.21 ^{bc}	8.61±0.89 ^b	13.89±1.08 ^b	14.29±1.03 ^b	6.20±0.37 ^b	5.10±0.79 ^{bc}	6.56±0.68 ^a	0.70±0.02 ^b	88.80±3.78 ^b
4 hr	102.32±6.01 ^a	9.46±0.99 ^a	15.46±1.90 ^a	18.09±1.40 ^a	6.60±0.60 ^{ab}	5.74±0.84 ^b	5.48±0.48 ^{abc}	0.78±0.02 ^a	83.40±4.85 ^c
6hr	83.20±5.30 ^c	6.83±0.70 ^c	12.94±0.46 ^{bc}	11.51±0.96 ^c	5.80±0.86 ^c	5.40±0.35 ^{bc}	6.04±0.80 ^b	0.70±0.03 ^b	84.00±4.03 ^{bc}
8hr	67.92±4.30 ^d	6.42±0.54 ^c	12.03±0.81 ^c	12.03±1.04 ^c	5.40±0.81 ^c	5.14±0.61 ^c	4.62±0.75 ^c	0.56±0.04 ^c	84.60±4.00 ^{bc}
Control	98.43±6.29 ^b	9.40±0.89 ^a	15.18±2.04 ^a	15.28±1.15 ^{ab}	7.20±0.58 ^a	6.10±0.44 ^a	6.42±0.26 ^a	0.76±0.13 ^a	92.60±4.12 ^a

Values with same alphabet are not significantly different (P<0.05) along the column.

Key: TRT = Treatment, PH = Plant height, NTP = Number of tiller/plant, NLT = Number of leaf/tiller, LL = Leaf length, NPT = Node/plant/tiller, IL = Internode length, LSL = Leaf sheath length, LB = Leaf breadth, NDM = Number of days to maturity.

Table 3: Quantitative seed characteristics (at maturity) of *D. exilis* treated with 0.1M nitrous acid.

Treatments	Spike/plant	spikelet/spike	spikelet/plant	% seed set	100-grains (g)
2 hr	3.21 ^b ±0.56	89.1 ^b ±16.4	462.0 ^c ±87.5	85.2 ^a ±15.0	0.094 ^{ab} ±0.6
4 hr	3.64 ^a ±0.51	91.8 ^a ±14.9	524.6 ^a ±81.9	85.4 ^a ±7.9	0.124 ^a ±0.5
6 hr	3.02 ^b ±0.63	66.6 ^c ± 19.8	315.0 ^{bc} ±58.6	79.0 ^{ab} ±15.9	0.089 ^{abc} ±0.4
8 hr	2.97 ^b ±0.43	56.2 ^d ±20.4	257.2 ^{abc} ±83.9	74.2 ^b ±9.1	0.082 ^c ±0.5
Control	3.51 ^a ±0.58	90.24 ^a ±15.4	485.2 ^b ±47.6	82.2 ^a ±13.9	0.105 ^b ±1.0

Values with same alphabet are not significantly different ($P < 0.05$) along the column.

Table 4: Qualitative characteristics of *D. exilis* treated with 0.1M nitrous acid

Treatments	Growth pattern (at maturity)	Colour at maturity	Pubescence
2hr	Erect	1	Absent
4hr	Erect	1	Absent
6hr	Semi-erect	2	Absent
8hr	Strangling	2	Absent
Control	Semi-erect	1	Absent

Note: 1 = Green stem base, green leaf sheath and pale green leaf.
2 = Reddish stem base, reddish-green leaf sheath and dark green leaf.

DISCUSSION

The quantitative and qualitative characters evaluated showed distinct differences. Seed colour, texture and shape were the same before and after the treatments, at harvest, the seeds (grains) remained the same for such characters, which suggests that the treatments produced no physical effects on the seed morphology of the studied crop. Different trends were recorded on the germination and percentage viability of the treated seeds. Though 2 hr exposure of seeds to 0.1M nitrous acid improved speed of germination, it had low percentage of germinated seed when compared with the control. The observed reduction in seed germination among mutagenic plants may likely be due to delay in physiological and biological processes necessary for seed germination which includes enzyme activities, hormonal stimulation and mitotic process (Ananthaswamy *et al.*, 1971; Khan and Goyal, 2009). A similar pattern of decrease in germination and seedling survival with increase in concentration of mutagenic agents was reported by different workers (Mensah *et al.*, 2007; Micco *et al.*, 2011; Shaguta *et al.*, 2013).

Low rate of survival observed after seedling transplant of 6 hr and 8 hr plants, may possibly be due to adverse effect of high concentration of nitrous acid on growth hormones. The 6 – 8 hr of seeds exposure to nitrous acid had impaired the *de novo* synthesis which adversely affected recovery from transplant shock, root formation and subsequent development of the plant as opined by Sato and Gaul (1967). In addition, decrease in seedling survival has been attributed to physiological disturbances or chromosomal damage caused to the cells of the plant by the mutagen. More also, Adegoke (1984) had reported that mutagens are capable of inducing chromosomal damage leading to bridge formation during mitotic division and hence increased phenotypic aberrations.

The results showed different degrees of response by the plant to mutagenic effects of nitrous acid. Quantitative growth parameters were significantly affected by exposure time. Four (4) hr of exposure to nitrous acid induced optimal vegetative growth but longer time of treatment adversely affected growth of *D. exilis*. Similar increases in growth parameters have been reported by Mensah *et al.* (2007) on *Sesamum indicum* using sodium azide. Analysis of data showed that significant early maturity was achieved with all regimes of treatments evaluated in this study which agreed with previous reports on utilization of mutagenesis to achieve early maturity in plants (Shamusuzzaman *et al.*, 2005; Mensah *et al.*, 2007).

In comparison with the control plants, the most efficient treatment time for induction of variation in *D. exilis* using 0.1M nitrous acid is 4 hr exposure of seeds. It induced highest number of spikelets and grain formation and performed optimally for all fruiting characters evaluated including 100 grains weight. Biswas and Datta (1988) worked on *Trigonella foenumfraeum* treated with 0.25% E.M.S and concluded that number of branches, pod per plant and other yield parameters were improved by mutagenesis. Discrepancy in plant coloration at emergence and maturity was earlier reported by Ukwungwu *et al.* (2003) who observed different plant colouration at juvenile and maturity stages in rice. Ability of plants of 2 and 4 hr treatments to produce erect plants suggests that nitrous acid could be utilized to improve logging which is a wild character of *D. exilis* that constitutes a barrier for mechanical harvesting of the crop.

CONCLUSION

Although germination and seedling survival were mutagenic dosage dependent with higher treatment period or concentration affecting adversely the

growth and yield of *D. exilis*, a shorter time of treatment of 4 hr produced significant variability and desirable effects on the crop. Generally for all the characters evaluated, 4 hr treatment consistently produced optimal effect on the crop. Nevertheless, all the treatment time evaluated was suitable for inducing and creating early maturing mutant of *D. exilis*. Treatments of 2 and 4 hr produced erect plants which could further be screened and improved upon to enhance mechanical harvesting of the crop subsequently making the crop appealing for large scale cultivation. Therefore, mutagenesis using nitrous acid could be explored further for production of improved *D. exilis* varieties.

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