Research Article

# Chemo-sensitivity and Determination of Optimum Exposure Time of Nitrous Acid for Growth and Yield of *Digitaria exilis* (Haller)

ANIMASAUN DA.*, MUSTAPHA OT., OYEDEJI S., YUSUF KA.
Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, P. M. B. 1515, Ilorin, 240003, Kwara State, Nigeria

## ABSTRACT

In this study, we assessed chemo-sensitivity of *Digitaria exilis* to nitrous acid in term of growth and yield to determine the mutagenic effects and optimum exposure time for desirable mutagenesis that may be utilize in the crop improvement. We exposed dried seeds of *D. exilis* to 0.1 M nitrous acid for varying time of 2, 4, 6, and 8 hours while 0 hr seeds were used as control. The seeds were sown and effects of exposure time were studied on the quantitative and qualitative characters. Results showed germination and seedling survival decreased with increased in exposure time. Data analysis revealed that 6 and 8 hr of treatment negatively affect plant height, number of leaves, tiller/plant, and leaf length. Though, all exposure time induced significant early maturity, however, 6 and 8 hr exposure produced low seed set and grain yield. The performance of the chemical treated plants was optimal for 4 hr exposure time which produced best agronomic traits, a degree of variability and improved yield. Also, 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 4 hr of exposure of *D, exilis* to 0.1M nitrous acid could be utilized in inducing variability and improvement of the crop in a breeding programme.

**Keywords:** Chemosensitivity, *Digitaria exilis*, Exposure time, Nitrous acid

## 1. INTRODUCTION

Among the dominant cultivated grasses of the tropic and the sub-tropic regions is Digitaria, a member of Poaceae family which consists of about 300 species of both annual and perennial grasses [1]. 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 [2]. *D. exilis* is the most important 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 Dominic Republican [3]. The grass is a small-seeded cereal that has great genetic developmental potential, its long shelf life and grain keeping qualities are particularly of importance in its potential for replacing the super cereals crops in industrial process for food production of tomorrow [4].

***Corresponding Author - Animasaun DA**. Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, P. M. B. 1515, Ilorin, 240003, Kwara State, Nigeria. E-mail -animasaun.ad@unilorin.edu.ng, biostanleydayor@yahoo.com.au
Received –12/10/2015 Accepted – 27/09/2016

Among the Hausas in Nigeria, Benin, Togo and Ghana, acha is made into a special conspicuous food types 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 documented [3- 6]. 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 150–200Kg/ha on a very poor soil [7]. The small 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 [7, 8].

The use of mutagenesis in crop improvement has led to the development of several improved varieties of economic values and further increased the understanding of genetics and plant developmental phenomenon. Enormous variability and extended gene pool has been created and established by induced mutagenesis of crop plant germplasm on which further selection and screening had resulted in creation of cultivars or varieties of novel characteristics. In the recent time, chemical mutagens have become important tools in crop improvements. These mutagens are used to develop disease resistance, abiotic resistance, high yield and nutrient fortified varieties. A number of workers have studied mutagenesis of different crops [9-11], but very scanty information was available on mutagenesis application in acha for the crop improvement [12].

In view of very little information available on the improvement of *D. exilis* by mutagenesis, this work aimed at assessment of chemosensitivity of *D. exilis* to nitrous acid to determine optimum exposure time for better agronomic and yield performances of the crop and to create variability on which selection could be based for the purpose of breeding and improvement.

## 2. MATERIALS AND METHODS

### 2.1. Seed material and treatment

*D. exilis* seeds used for this study were collected from the Acha Breeding Unit, National Cereal Research Institute, Badeggi, Niger State of Nigeria. Ten grams of clean and dried seeds were weighted into four separate petri-dishes and treated with freshly prepared Nitrous acid (0.1M) for varying times of 2hr, 4hr, 6hr and 8hr. After the treatment time, the 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 experimental control seed were soaked in distilled water for 3 hr and then air-dried prior to sowing.

### 2.2. Pot experiment and sensitivity evaluation

Seeds were sown in nursery beds rich in loamy soil (0.5m x 0.5 m) prepared for each of the treatment time and the control in the Biological Garden, University of Ilorin, (N $08^0$ 28' 53.3'', E $04^0$ 40' 28.9"), Ilorin, Nigeria. Appropriate cultural practices were carried out when due. On the third week after sowing, three plants were transplanted into a five liter capacity planting bag filled with soil and labeled according to the seedlings treatments. Five replicates were made for each of the treatments and control in a complete randomized block design.

Vegetative characters were evaluated 3 weeks after transplanting and at maturity. Characters on which effect of treatments were evaluated are 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. Yield characters considered are; number of days to maturity, number of 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, which was determined using Mettler balance (Model PL 300). Five different measurements were taken in all cases and the means were evaluated.

**Table 1: Quantitative characteristics of *D. exilis* treated with 0.1M nitrous acid at different exposure time at 3 weeks after transplanting**

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

**Table 2: Quantitative characters of *D. exilis* responses to 0.1M Nitrous acid of different exposure time**

| 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$^{abc}$ |
| 6hr | 83.20±5.30$^{c}$ | 6.83±0.70$^{c}$ | 5.94±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$^{c}$ |
| 8hr | 67.92±4.30$^{d}$ | 6.42±0.54$^{c}$ | 12.03±0.81$^{ab}$ | 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$^{c}$ |
| Contl | 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 difference along the column while values followed by different alphabets are significantly different at $P \leq 0.05$. 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 different time of exposure to 0.1M Nitrous acid**

| Treatments | Spike/plant | spikelet/spike | spikelet/plant | % seed set | 100-grains (g) |
|---|---|---|---|---|---|
| 2 hr | 3.21±0.56$^{b}$ | 89.1 ±16.41$^{b}$ | 462.0±87.56$^{c}$ | 85.2 ±15.05$^{a}$ | 0.094±0.61$^{ab}$ |
| 4 hr | 3.64±0.51$^{a}$ | 91.8 ±14.93$^{a}$ | 524.6±81.91$^{a}$ | 85.4 ±7.92$^{a}$ | 0.124±0.53$^{a}$ |
| 6 hr | 3.02±0.63$^{b}$ | 66.6 ± 19.81$^{c}$ | 315.0±58.64$^{bc}$ | 79.0 ±15.94$^{ab}$ | 0.089±0.44$^{abc}$ |
| 8 hr | 2.97±0.43$^{b}$ | 56.2 ±20.47$^{d}$ | 257.2±83.92$^{abc}$ | 74.2 ±9.11$^{b}$ | 0.082±0.51$^{c}$ |
| Control | 3.51±0.58$^{a}$ | 90.24 ±15.41$^{a}$ | 485.2±47.62$^{b}$ | 82.2 ±13.93$^{a}$ | 0.105±1.06$^{b}$ |

Values with same alphabets are not significantly difference along the column while values followed by different alphabets are significantly different at $P \leq 0.05$

Plant colour was observed at 3week after emergence, while growth pattern and pubescence were observed at maturity.

## 2.3. Data analysis

Data obtained from the evaluated quantitative growth and yield parameters were subjected to analysis of variance (ANOVA) using SPSS Version 16.0. The means were separated with Duncan Multiple Range Test (DMRT) at the probability level of $P \leq 0.05$.

## 3. 4. RESULTS

Germination characteristics of *D. exilis* in response to varying nitrous acid exposure time are shown in Fig 1a and 1b. Germination commenced on the 4$^{th}$ day after sowing. Mean days to emergence for the control seeds were 6.2 days, with percentage germination of 90.3%, while mean days to emergence for the nitrous acid treatments

**Table 4: Qualitative characteristics of *D. exilis* at maturity in response to difference exposure time of 0.1M Nitrous acid**

| Treatments | Growth pattern | 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 = Reddishstem base, reddish-green leaf sheath and dark green leaf.

ranged between 4.8-7.8 days. The average seedling emergence for 2 hr treated seeds was 4.8 days with percentage germination of 78.18%. Meanwhile, the4 hr treated-seeds emerged 6.2 days after

sowing with the highest percentage germination (81.12%) among the treated seeds. In contrast, the6hr and 8hrhadbetween 7-8 days to germination and very low percentage seed germination. The percentage survival of the seedlings after transplant also decreased with increased in treatment time. While 83.3 and 80.4% of 2 hr and 4 hr seedlings survived respectively after transplant, only 40% of 6 hr seedlings and 44% of 8 hr seedlings survived, the remaining ones died few days after transplanting. However, in comparison with control, seedling survival after transplant was 94.3% (Fig. 1a, b).

Vegetative quantitative traits considered 3 weeks after sowing showed number of leaves per plant, was highest (3.2) in 2 hr plants but they was not statistically different from the control (3.1). Leaf length was similar for 4 hr and control while 2hr and 8 hr plant had significantly shorter leaf blade (Table 1). In term of leaf breadth, 4 hr and control plant were at per, but narrower leaves were obtained with other treatment exposure durations. Plant height was highest (3.92 cm) in the 4 hr treated plant which was similar to the control plant (3.82 cm), average plant height for 2 hr was 3.14 cm, however, higher treatment exposure time of 6 and 8 hr induced less vegetative growth (Table 1).

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

The fruit and yield characteristic of *D. exilis* in response to treatments are presented in (Table 3). Fruit set, yield 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. The 2hr and 4hr nitrous acid-treated plants had reddish stem base and reddish-green leaf sheaths with pale green leaf, while 6hr and 8hrplantshad 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.

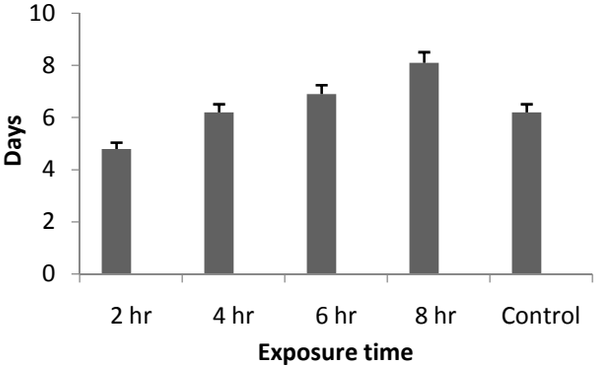


**Figure 1a: Mean number of days to germination of *D. exilis* treated with Nitrous acid at different exposure time.**

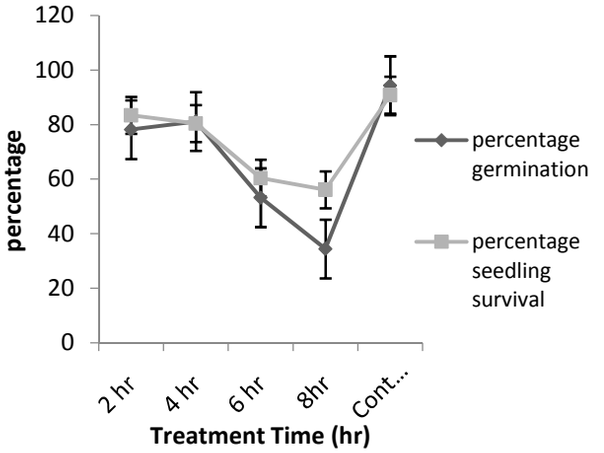


**Fig 1b: Percentage germination and seedling survival of *D. exilis* in response to different time of exposure to 0.1M nitrous acid.**

## 4. DISCUSSION

The quantitative and qualitative characters evaluated showed varied chemosensitive responses of *D. exilis* to different exposure time of nitrous acid. Seed colour, texture and shape are the same before and after the treatments even the $M_1$ seeds remained the same for such characters, which sug-

gests that the treatments produced no physical effects on the seed with respect to those characters. Different trends were recorded on the germination and percentage viability of the treated seeds. The observed reduction in seed germination among mutagenic plants may be due to mutagenic sensitivity of the seed which caused delayed physiological and biological processes necessary for seed germination; these includes enzymes activities, hormonal stimulation and mitotic process [11, 12]. A similar pattern was reported by different workers that germination and seedling survival decreases as mutagenic concentration increases [12-15].

Low rate of survival observed after seedling transplant of 6hr and 8hr plants, may possibly be adduced to decrease in synthetic process of the DNA and RNA [16]. Conversely, 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,[17.] In addition, the decrease in seedling survival could be attributed to physiological disturbances or chromosomal damage caused to the cells of the plant by the mutagen. The present finding concurred with a report that demonstrated that mutagen induces chromosomal damage leading to bridge formation during mitotic division and hence increased phenotypic aberrations which could affect plant growth and survival [18].

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, whereas 4 hr of exposure to nitrous acid induced optimal vegetative growth, longer time of treatment adversely affected growth of *D. exilis.* Similar increase in growth parameters have been reported on *Sesamum indicum* using Sodium azide [10]. Analysis of data showed that significant early maturity was achieved with all regimes of treatments evaluated in this study which concurred with previous reports on utilization of mutagenesis to achieve early maturity in plants [12, 14, 19 20]

In comparison with the control plants, the most efficient treatment time for induction of mutation in *D. exilis* using 0.1M nitrous acid is 4 hrof seed exposure. It induced highest number of spikelets and grain formation and performed optimally for all fruiting characters evaluated even 100 grains weight. The present work is in tanderm with earlier report on *Trigonella foenum fraeum* treated with 0.25% E.M.S which concluded that number of branches, pod per plant and other yield parameters were improved by mutagenesis [16]. Discrepancy in plant coloration at emergence and maturity as observed in this present work had been earlier reported [21]. The occurrence and disappearing of stem base colouration in higher exposure treated plants indicated that longer exposure to nitrous acid may cause mutation of chlorophyll at juvenile which get normalized or permanent at maturity. To corroborate this, higher dose of physical mutagen (Gamma radiation) has been implicated in stem base colouration variation in *D. exilis* [12]. Ability of mutagenic 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 prevents and constitute a barrier for mechanical harvesting of the crop.

## 5. CONCLUSION

Although germination and seedling survival were sensitive to chemical exposure regimes, longer exposure time adversely affected growth and yield of *D. exilis*. Moderate exposure time of treatment of 4 hr produced significant positive 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 and subsequently making the crop appealing for large scale cultivation. Therefore, mutagenesis sensitivity of nitrous acid could be explore further and utilized for breeding improved *D. exilis* varieties.

## 6. ACKNOWLEDGMENT

The authors are grateful to Dr. Maji, A. T of Acha Breeding Unit, National Cereal Research Institute, Badeggi, Niger State of Nigeria for providing the seed materials for this study

## 7. REFERENCES

1. Bodgan AV. Tropical pastures, fodder plants (Tropical Agriculture series) Longman Inc. New York. 1977. pp 216-244.
2. Kativu S. Mhlanga L.Zimbabwean grasses of cereal value. Plant Genetic Resource Newsletter. 1995.101: 15-19
3. Morales-Payan JP. Ortiz JR. Cicero J. Taveras F.*Digitaria exilis* as a crop in the Dominican Republic. In: Janick, J. and Whipkey, A. (eds.), Trends in new crops and uses. ASHS Press, Alexandria, VA. 2003. p 140-153
4. Dachi SN and Gana AS. Adaptability and yield evaluation of some Acha (*Digitaria exilis* and *Digitaria iburua* Kippis Stapf) accessions at Kusogi – Bida, Niger State, Nigeria. African Journal of General Agriculture. 2008. 4(2): 73 - 77
5. Jean-Francois C. Fonio: a small grain with potential. ELISA Magazine. 2004. 20(1): 1-4
6. Kwon-Ndung E.Dachi S. Ukwungwu M. Imolehin ED. Potential role of Biotechnology tools for genetic improvement of 'lost crops of Africa': the case of fonio (*Digitaria exilis* and *Digitaria iburua*), African Journal of Biotechnology. 2003. 2(12): 580-585.
7. Maji AT. Dachi SN. Yisa J. Evaluation of morphological variations within and between Acha collections in NCRI accessions. NCRI Annual Report.2003. pp, 90-94
8. Maji AT. Dachi SN. Yisa J. Improvement of Acha accession at NCRI, Badeggi, NCRI Annual Report. 2005. pp. 83-93
9. Nilan RA. Kleinhofs A. Konzak, CF. The role of induced mutation in supplementing natural genetic variability. Annals of New York Academy of Science. 1977. 287: 367-384.
10. Mensah JK. Obadoni BO. Akomeah PA. Ikhajiagbe B. Ajibulu J. The effects of sodium azide and Colchicine treatments on morphological and yield traits of sesame seed (*Sesame indicum* L.). African Journal of Biotechnology. 2007. 6(5): 534-538.
11. Chowdhury R. Tah, J. Assessment of chemical mutagenic effects on mutation breeding programme of M1 generation of Carnation (*Dianthus caryphyllus*). Research in Plant Biology. 2011. 1(4): 23-32
12. Animasaun DA. Morakinyo JA. Mustapha OT. Assessment of the effects of gamma irradiation on the growth and yield of *Digitaria exilis* [Haller]. Journal of Applied Biosciences. 2014. 75:6164–6172
13. Ananthaswamy H.M. Vakil VK. Shrinivas A. Biological and physiological changes in gamma irradiated wheat during germination. Radiation Botany. 1971. 11: 1-12
14. Khan S. Goyal S. Improvement of Mungbean varieties through induced mutation. African Journal of Plant Science. 2009. 3: 174-180
15. Micco VD. Arena C. Pignalosa DDM. Effect of sparsely and densely ionizing radiation on plants. Radiation, Environmental and Biophysics. 2011. 50: 1-19
16. Shagufta B. Aijaz AW. Irshad AN. Mutagenic sensitivity of gamma rays, EMS and sodium azide in *Trogonella foenum-graecum* L. Science Research Reporter. 2013. 3(1):20-26
17. Sato M. Gaul H. Effect of EMS on fertility in Barley. Radiation Botany. 1967. 7: 7-10
18. Adegoke JA. Bridge induction by sodium azide in *Allium cepa.* Nigerian Journal of Genetics. 1984. 5: 86
19. Shamsuzlaman KM. Islam MM. Muwara B. Abdus S. Development of early maturing chickpea variety BINASOLA-3.Mutation Breeding Newsletter Review. 2005. 1*:* 10-13.
20. Animasaun DA. Oyedeji S. Azeez MA. Onasanya OA. Alkylating efficiency of sodium azide on pod yield, nut size and nutrition composition of samnut 10 and samnut 20 varieties of groundnut (*Arachis hypogea* L.). African Journal of Food, Agriculture, Nutrition and Development.2014. 14(7): 9498-9510
21. Ukwunguwu MN. Bakare SO. Kuta DD. Dachi SN. Evaluation of accessions of Acha in Badaggi. NCRI Annual Report. 2003. 148-157.