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Research Article

Selection of Drought Tolerant Mutant of *Amaranthus Cruentus* L. in Green House

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Water deficit is one of the significant environmental factors limiting plant production. This stress occurs during drought period. The aim of this study was to select gamma irradiation-induced drought tolerant mutant drought lines during vegetative stage. Seeds of the reference cultivar were gamma irradiated using the gamma irradiated facility at the IAEA to induce genetic variation. Four selected lines based on their high biomass (L_2, L_{17}, L_{18} and L_{23}) and the reference cultivar (L_0) were grown under four irrigation frequencies: 2, 4, 8 and 12 days with 200 ml as standard water regime for 24 days after transplanting. Completely randomized block design with 3 repetitions was applied. Data related to Relative Plant Height Growth, Relative Leaf Number Growth, Relative Shoot Fresh Mass Growth and Relative Root Length Growth were determined. The results indicated a significant effect of water deficit on all considered parameters by limiting the growth. Reduction observed were less accentuated in lines L_2. Line L_2 showed a significant difference for root growth indicating the adaptability of drought. Based on drought tolerant index, L_2 was the most tolerant. At this stage, lines L_2 appeared to be the most promising for the creation of new drought-tolerant amaranth varieties.

Keywords: *Amaranthus cruentus*, irrigation frequencies, mutant lines of amaranth, water deficit, tolerant lines.

INTRODUCTION

Leafy vegetables are important sources of both bioactive compounds and micronutrients for local populations in Sub-Saharan Africa (Smith and Eyzaguirre, 2007). Amaranth (*Amaranthus* spp) is a traditional vegetable produced for the leaves and seeds eaten in various forms (Mburu *et al.*, 2012). In Benin, it is one of the most produced and widely consumed vegetable crops (Achigan-Dako *et al.*, 2013). Amaranth species can be a major source of income, especially 89% were consumed in urban and peri-urban areas of Benin (Chouti *et al.*, 2018). The leaves are rich in protein, vitamin C, beta-carotene, iron, and calcium (Trucco and Tranel, 2011; Mburu *et al.*, 2012; Agre *et al.*, 2016). According to Achigan-Dako *et al.* (2013) and Agre *et al.* (2016), there are several species of amaranth in Benin but *Amaranthus cruentus* is the most cultivated and most consumed species. Its leaves are used for sauce and local private entrepreneurs pre-process the leaves into the dried and frozen precooked forms (Agre *et al.*, 2016).

In Benin, amaranth production is confronted with two major stresses: salinity and drought. Most Amaranth producers are located in the coastal areas closed to the sea, where the plant growth is affected by salinity at all stages of development leading a serious reduction in the yields (Kpinkou *et al*., 2018). Areas not affected by salinity exist in the country where producers are faced with water stress. This situation often leads them to abandon the poor lands and convert to other activities. As for *Amaranthus cruentus*, despite its popularity in Benin, it remains a neglected and underutilized species by both research and development, making it vulnerable to environmental constraints. For this species, no improved varieties are currently available.

Climate change is expected to contribute to severe drought in the Sub-Saharan African region which is already vulnerable to the Atlantic monsoon (Lézine *et al.,* 2011). Such drought stress contributes to reducing the photosynthesis in vegetables, hence seriously hampering the crop productivity (Dijkstra *et al.*, 2015; Abobatta, 2019). Drought also reduces the availability and transport of minerals. Consequently, the plant uptake is decreased sharply (Lemaire and Denoix, 1987; Meisser *et al.*, 2018). Furthermore, in tropical areas with an average of 2,600 mm of annual precipitation, the soil water reserve can decrease sharply during the dry season and limit plant growth and productivity (Cornic and Massacci, 1996; Tshiabukole *et al.*, 2017). The extent of yield loss depends on the type of soil, climatic conditions (interval between rains, intensity of stress), operating practices and type of vegetation (Gilgen and Buchmann, 2009; Meisser *et al.*, 2013; Meisser *et al*., 2018). The selection of drought-resistant varieties is an urgent issue to secure the availability of *A. cruentus* in Benin.

The genetic variability of *A. cruentus* is low in Benin and it is difficult to identify local varieties possessing strong drought resistance used for parental materials for breeding. We adopted the approach of mutagenesis to induce genetic variation in *A. cruentus* species. This research program supported by FAO/IAEA (Food and Agriculture organization/International Atomic Energy Agency) aimed to select tolerant lines to salinity for producers in the coastal areas and drought tolerant lines for growers inside the country.This study explored such possibility to develop superior genotypes that will be used to improve amaranth production inside the country for food and nutrition.

MATERIAL AND METHODS

Plant material

Induced mutations in Amaranth breeding program started in 2015 in Benin with the FAO/IAEA breeding program. The seeds obtained after the Fourth generation namely L_2, L_{17}, L_{18} and L_{23} and a local check (L_0) were used in this experiment. The local cultivar was used as parental material in the breeding program. The induced mutation was made by gamma irradiation on seed treatments at 200 grays using IAEA facility. Previous studies revealed that local cultivar used as control check is susceptible to abiotic stress including salinity (Wouyou *et al.*, 2017) and drought (Hegbe, 2020).

Experimental area

The experiment was carried out in the greenhouse of National Institute for Agricultural Research of Benin located at the latitude 6º4’ and longitude 2º3’using GPS. Plants were grown at a temperature of 26/22 ºC day/night under a constant relative humidity of 55%. The experiment performed in Abomey-Calavi. The city is in subequatorial climate and the average of temperature is 27.2°C and the annual precipitation is 1179 mm.

Experiment management

The experiment was setup in two steps. The first step was performed to determine the frequency of irrigation which can indicate the water deficit effect on the reference cultivar (Local). The soil used contains a mixture of sterilized sand and compost. Sixteen (16) seedlings were then transferred into pre-prepared pots 11.3 cm in diameter and 14 cm high containing 2/3 sand and 1/3 compost. Three (3) irrigation frequencies were applied, 2 days, 4 days, and 6 days. The water regime is 200 ml for all irrigation frequencies. The second step used the seeds of the four lines and the check cultivar. Incubation of seeds in trays was done for three weeks. The germination and transplanting conditions are the same as in both steps. One hundred and eighty (180) young plants were then

transferred to the pots 21 days after germination. Thus, the 4 mutant lines of amaranth (*Amaranthus cruentus*) and the reference cultivar were installed. The pots were kept clean by manual weeding whenever necessary. The evaluation of their behavior in response to water deficit for 24 days, began a week after transplanting for their resumption. Four irrigation frequencies were applied, a normal frequency of 2 days and three stressful frequencies of 4 days, 8 days, and 12 days. The water regime was the same i.e. 200 ml. Randomized Complete Block Design was performed with 3 replications.

Data collection

Plant height, leaf number, shoot fresh mass and root length were measured before stress application (X_0). The same parameters were measured again after drought application (X_1).

Relative Plant Height Growth (RPHG), Relative Leaf Number Growth (RLGN), Relative Shoot Fresh Mass Growth (RSFMG) and Relative Root Length Growth (RRLG) were calculated as (Wouyou *et al*., 2017):

$$\frac{X_1 - X_0}{X_0} \quad (1)$$

Data analysis

To test the effect of irrigation frequencies on genotypes using growth parameters, linear mixed model was performed with the *lmerTest* package (Kuznetsova *et al.*, 2017). Replication was a random factor while genotypes and irrigation frequencies were considered fixed. Analysis of variance (ANOVA) and the means were compared with the Tukey-Kramer test. The growth parameters were then regressed by the irrigation frequencies to relate them. Fernandez (1992) formula was used to determine the stress tolerance index (STI). It calculated for each parameter. Two days irrigation frequency is the control

$$STI = \frac{Y_s \times Y_p}{\bar{Y}_p{}^2} \quad (2)$$

Ys mean the measurement under control experiment
Yp mean the measurement under stress

RESULTS AND DISCUSSION

Water deficit x genotypes

Results of the two-ways analysis of variance indicated significant interaction ($p<0.05$) water deficit x Genotypes effect for Relative Plant Height Growth (RPHG) and Relative Shoot Fresh Mass Growth (RSFMG; TABLE 1). Interactions were not significant ($p>0.05$) for other variables as Relative Leaf Number Growth (RLNG) and Relative Root Length Growth (RRLG). Genotype effects showed significant for RLNG, RSFMG, RRLG.Water deficit exhibits significant effect for all considering parameters interclass correlation coefficient (ICC) obtained were 0; 0; 0.3924 and 0.3189 for RPHG; RLNG; RSFMG and RRLG respectively indicating low homogeneity among classes. ICC close to zero means no homogeneity/similarity for values from the same class. According to Achigan-Dako *et al.* (2013) *Amaranthus* sp was found as a promising food crop, mainly due to its resistance to heat, droughts, diseases and pests, and the high nutritional value of its seeds and leaves. *Amaranthus cruentus* is highly tolerant to unfavorable environmental conditions as poor soils, lack of water and severe defoliation (Parra-Cota *et al.*, 2014). Moreover, water deficit is a major problem that affects plant production. It causes changes in the expression of many genes (Gaufichon *et al.*, 2010).

Effect of water deficit on each variable

Plant Relative Height Growth

Local control (L_0) showed significant difference for plant height ($P<0.0003$). This result indicated a decrease of Plant Relative Height Growth (PRHG) across the different irrigation frequencies (2, 4, 8, 12 days). PRHG varied from 0.636 to 0.138. L_2 mutation line revealed 0.458 at 2 days and 0.232 as PRHG values at 12 days and no significant effect was observed ($P=0.1216$). The three mutation lines as L_{17}, L_{18} and L_{23} showed similar tendency. Irrigation frequencies had a negative effect on plant relative height growth. As the stress effect becomes severe, PRHG values decrease. This result is shown by the significant probabilities (TABLE 2). From these results, PRHG was similar for all irrigation frequencies for L_2. This line appears to be drought tolerant. Application of the four water regimes on the five genotypes revealed impact of drought on *Amaranthus cruentus* growth and the critical plant development phases during which plants are more vulnerable. Similar results were reported by Attia (2007) on cotton and Tshiabukole *et al.* (2017) on maize.

Relative Shoot Fresh Mass Growth

In our condition, shoot fresh mass growth is very important. It is the commercial part for amaranth. Local cultivar (L_0), lines L_{17}, L_{18} and L_{23} indicated variable relative shoot fresh mass growth (RSFMG) from 2 to 4 days irrigation frequencies. Therefore, it was not significantly different from 2 to 8 days irrigation frequencies for line L_2 (TABLE 3). For L_2, only 12 days irrigation frequency showed a decreased relative shoot fresh mass growth. Probably, L2 limited water lost during transpiration when water deficit is severe. The applied water stress caused reductions in the dry matter accumulation in *A. cruentus* species studied, being the lowest irrigated treatment (Silva *et al.*, 2019).

Relative Leaf Number Growth

Lines L_0, L_{17}, L_{18} and L_{23} show significant Relative Leaf Number Growth relative to irrigation frequencies (TABLE

4). Any variability was not observed from 2 to 12 days for L_2 line. Number of leaves is linked to shoot fresh mass. This is due to the fact that plants were stressed due to lack of water and tend to perform a lower rate of cell division; thus reducing leaf production, providing a lower accumulation of dry matter at the end of the cycle (Souza and Amorim, 2009 ; Silva and Nogueira, 2003). Similar observation was made by Wouyou *et al.* (2017) working in salt stress on *A. cruentus*.

Relative Root Length Growth

The growth of root length allows plant to seek water for metabolic functions. Relative Root Length Growth (RRLG) indicated significant difference for L_0, L_{17}, L_{18} and L_{23} except L_2 between 8 days and 12 days irrigation frequencies (TABLE 5). Irrigation frequencies have no effect on root length for line L_2 when drought is severe. L_2 demonstrated the ability to drought tolerance. Root growth with water restriction was altered *A. cruentus*. Increasing the amount of root during drought helps the plant to obtain water at deeper levels in the soil profile, as well as helping to avoid water deficits in the more superficial layers of the soil (Ludlow and Muchow, 1990; Silva *et al.*, 2019). If water has been available, the plants would concentrate their roots in the superficial layers, where the growth is easier (Silva *et al.*, 2019).

Drought Tolerance Index

Number of leaves and the fresh mass are the two important parameters for the producer. Their index will allow you to choose the tolerant line. Considered the two parameters, L2 presented the high values of drought tolerance index (TABLE 6). Then L_2 appear to be the tolerant line. Based on drought tolerance index, Wouyou *et al.* (2017) identified among *A. cruentus* species, the Red cultivar as a salt tolerant cultivar. Moreover, the drought tolerant varieties were selected by Tshiabukole *et al*. (2017) among the cultivars in RD Congo.

CONCLUSION

Water deficit causes a significant reduction in the growth parameters considered in this study. The four lines tested show variable behavior depending on each line. However, lines 2 the most drought tolerant while the local check is the most sensitive. Among the mutated lines, L_2 can be selected as a drought tolerant line.

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COMPETING INTERESTS

Authors declare that no competing interests exist

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Table 1: Combined analysis of Genotypes and Irrigation frequencies on growth parameters

Parameters	Statistics	Genotypes	Irrigation frequencies	ICC Replications
RPHG	F value	0.749	82.029	0
	Pr(>F)	0.562	<0.001	
RLNG	F value	3.980	73.982	0
	Pr(>F)	<0.001	<0.001	
RSFMG	F value	9.419	71.509	0.3924
	Pr(>F)	<0.001	<0.001	
RRLG	F value	3.034	70.607	0.3189
	Pr(>F)	0.025	<0.001	

RPHG: Relative Plant Height Growth; RLGN: Relative Leaf Number Growth; RSFMG: Relative Shoot Fresh Mass Growth;
RRLG: Relative Root Length Growth; ICC: Intraclass correlation coefficients

Table 2: Plant Relative Height Growth

Genotypes		Irrigation frequencies				
		2 Days	4 Days	8 Days	12 Days	Prob
L_0		0.636±0.018^{a}	0.373±0.062^{b}	0.285±0.043^{bc}	0.138±0.044^{c}	0.0003
L_2		0,458±0.096^{a}	0.443±0.058^{a}	0.250±0.034^{a}	0.232±0.091^{a}	0.1216
L_{17}		0.969±0.117^{a}	0.607±0.095^{b}	0.127±0.045^{c}	0.089±0.043^{c}	0.0002
L_{18}		0.686±0.114^{a}	0.344±0.065^{ab}	0.318±0.056^{b}	0.281±0.050^{b}	0.0183
L_{23}		0.722±0.127^{a}	0.546±0.077^{ab}	0.265±0.034^{bc}	0.097±0.018^{c}	0.0019

Table 3: Relative Shoot Fresh Mass Growth

	Irrigation frequencies				
Genotypes	2 Days	4 Days	8 Days	12 Days	Prob>F
L_0	1.739±0.288^{a}	0.347±0.100^{b}	0.152±0.062^{b}	0.086±0.057^{b}	0.0002
L_2	1.298±0.428^{a}	1.187±0.493^{a}	0.442±0.413^{ab}	0.07±0.078^{b}	0.0128
L_{17}	3.785±0.851^{a}	1.4±0.385^{b}	0.407±0.017^{c}	0.381±0.030^{c}	0.0027
L_{18}	2.78±0.326^{a}	1.526±0.254^{b}	1.214±0.250^{b}	0.859±0.169^{b}	0.0037
L_{23}	4.163±0.645^{a}	2.442±0.385^{ab}	1.15±0.317^{b}	0.901±0.324^{b}	0.0028

Table 4: Relative Leaf Number Growth

	Irrigation frequencies				
Genotypes	2 Days	4 Days	8 Days	12 Days	Prob>F
L_0	0.6±0.115^{a}	0.2±0.01^{b}	0.133±0.066^{bc}	0.066±0.066^{c}	0.004
L_2	0.933±0.176^{a}	0.866±0.133^{a}	0.533±0.176^{a}	0.4±0.115^{a}	0.1056
L_{17}	1.4±0.115^{a}	1±0.115^{ab}	0.666±0.176^{bc}	0.2±0.115^{c}	0.0013
L_{18}	1.933±0.133^{a}	1.533±0.066^{ab}	1.466±0.133^{ab}	1.2±0.115^{b}	0.0131
L_{23}	2.066±0.176^{a}	1.733±0.176^{ab}	1.266±0.240^{b}	1.266±0.133^{b}	0.0412

Table 5: Relative Root Length Growth

	Irrigation frequencies				
Genotypes	2 Days	4 Days	8 Days	12 Days	Prob>F
L_0	1.511±0.119^{a}	0.852±0.277^{ab}	0.738±0.491^{ab}	0.363±0.223^{b}	0.0118
L_2	1.583±0.132^{a}	0.958±0.055^{b}	0.427±0.135^{c}	0.187±0.095^{c}	<.0001
L_{17}	1.175±0.092^{a}	0.5±0.162^{b}	0.25±0.096^{b}	0.074±0.037^{b}	0.0004
L_{18}	0.746±0.352^{a}	0.514±0.093^{a}	0.45±0.078^{a}	0.422±0.154^{a}	0.6733
L_{23}	1.466±0.245^{a}	0.65±0.052^{b}	0.625±0.190^{b}	0.266±0.158^{c}	0.0079

Table 6: Drought (stress) Tolerance index

	Paramètres			
Genotypes	RPHG	RLNG	RSFMG	RRLG

L_0	0.418±0.10	0.246±0.06	0.117±0.03	0.127±0.07
L_2	0.683±0.13	0.607±0.10	0.454±0.17	0.327±0.07
L_{17}	0.293±0.10	0.437±0.09	0.208±0.07	0.237±0.07
L_{18}	0.442±0.03	0.428±0.05	0.449±0.07	0.230±0.17
L_{23}	0.428±0.11	0.490±0.06	0.364±0.08	0.263±0.07

RPHG: Relative Plant Height Growth; RLNG: Relative Leaf Number Growth;
RSFMG: Relative Shoot Fresh Mass Growth; RRLG: Relative Root Length Growth