
RESEARCH ARTICLE

Development of female parent sorghum with high lysine and threonine content in Mali

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Abstract

Sorghum is poor in minerals, protein content and nutritional value for resource-poor farmers in Mali. It is therefore important to provide the population with high-yielding hybrids sorghum, more nutritious and adapted to the agro-ecological conditions of the country. From 2018 to 2020 seasons, activities were carried out at the research station of IER with a goal to identify maintainer lines containing high lysine and threonine content. However, twenty-six lines were maintainer of sterility with anthers color (white) and form (thin). Sixteen lines as partial maintainer of sterility or restorers of fertility. The color and form of anthers (BR lines) were plump and yellowish. Thirty-three lines were restorer of fertility (R lines). The color and form of anthers (R lines) were plump and yellowish. The nine B lines had a good grain qualities and endosperm textures. The grain weight was 101 to 375 g for all nine B lines. These nine B lines are worthy for developing sorghum hybrids containing high lysine and threonine content.

Key words: Maintainer, Restorer, Lysine, Threonine, Sorghum

Introduction

Sorghum is an important crop in Mali, yet grain yields are low and do not contain high lysine and threonine content. The development of F₁ hybrids with high lysine and threonine content could generously enhance grain yields and nutrients content as mentioned by Tefera (2020). Researchers have created methodologies to develop hybrid sorghums with high lysine content and protein concentrations (Axtell *et al.*, 1973). Sorghum has been described by Reddy *et al.*, (2006) as the first self-pollinated cereal staple crop, in which F₁ hybrids have been financially misused to enhance efficiency. This is because of the accessibility of a stable and heritable cytoplasmic male sterility (CMS) system as mentioned by (Stephens *et al.*, 1954). Use of CMS systems for hybrid improvement requires the advancement of restorer lines that restore pollen fertility to CMS lines. Kumar *et al.*, 2010 reported that the finding of cytoplasmic-nuclear male sterility (CMS) and genetic male sterility (GMS) has encouraged the utilization of choice strategies and hybrid development techniques in sorghum improvement.

As Dhillon *et al.*, (2008) pointed out that cytoplasmic male-sterility (CMS) has been effectively used to establish hybrid for expanding crop production around the world. The discovery of A1 (milo) CMS system by Stephens and Holland (1954) in sorghum made possible the development of hybrid. This specific wellspring of male sterility has been the prevalent wellspring of male sterility in hybrid sorghum for as long as 40 years mentioned by Rooney and Serna-Saldivar, (2003). As per Moran and Rooney (2003), all the hybrids delivered as currently and that are generally developed depend on the A1 (milo) cytoplasm. In reality some presently utilize the A4 CMS yet most are still on A1. Cytoplasmic Male Sterility framework is a physiological irregularity coming about because of discordant collaboration between the cytoplasmic elements, the mitochondrial hereditary elements and nuclear genetic components prompting the creation of degenerate or non-viable pollen grains or non-dehiscent anthers with or without useful pollen grains as discovered by Reddy *et al.*, (2004). As mentioned by Maunder and Pickett (1959) the sterility of the anthers shows when the plant has the cytoplasm “milo” and does not have the genes for fertility restoration, *rfrf*. The male-sterility results from the connection of male-sterile cytoplasm from the sorghum “milo race” and nuclear genes in the “kafir” race that neglect to reestablish male fertility as reported by Stephens and Holland (1954). Kafir was subsequently considered as a maintainer of the milo wellspring of CMS. The male sterile lines “A” is utilized as female parents in hybrid seed production. A line is not regarding milo race, however any sorghum that shows cytoplasmic-genic sterility. It is kept up by the isogenic line called “B” which has ordinary cytoplasm and produce fertile pollen yet do not have any nuclear fertility reestablishing genes. The B line is isogenic to the A line and, in light

of the fact that it lacks fertility restoring genes, it tends to be crossed onto the CMS A line and produce male sterile offspring. Recognizable proof of the promising new “A” lines is accordingly significant in breeding programs which expect to exploit Heterosis through high yielding hybrid production as reported by House (1985). At ICRISAT-Bamako, Mali, a few CMS restorer lines have been recognized by Reddy *et al.*, (2006) from a guinea germplasm. Various inter racial R-lines have been recognized by the IER-Mali program. Yet, these restorer lines are low in high digestibility/high lysine, a critical requirement influencing sorghum grain yield in Mali. However, in the sub-region, none females parent containing a high lysine and threonine content were developed.

Hence, the goal of this study was to identify maintainer lines (B-lines) rich in lysine and threonine concentration that would be valuable for developing hybrids for Southern Mali. Therefore, two specific objectives are to: Test the cytoplasmic restoration reaction of selected lines of F₁ populations derived from backcrossing (BC₁F₃) with cytoplasmic male sterility and Conversion of identified B-lines containing high lysine and threonine to A-lines. Hence, the current study was planned to identify the cytoplasmic restoration reaction of selected lines and then the conversion of B-lines containing high lysine and threonine to A-lines.

Materials and methods

This study was conducted at the Regional Center for Agronomic Research (CRRA) of Sotuba, IER Mali, is located in the district of Bamako. The climate is Sudano-Sahelian type. Coordinates are latitude 12°38', longitude 7°56' and an altitude of 320 m with rainfall varying from 800 to more than 1000 mm. The soil is of the clay loam or clay sandy type.

Test of cytoplasmic reaction of female parents (BC₁F₃)

The plant material consisted 75 successful F₁ hybrids from crosses between the female parent (12A) and 75 (BC₁F₃) from three populations (216-2BCTP1 // P851171, 150B // P851171 and 12B // P851171) (Table 1). The experimental design was a simple observation plot. The sowing was made on a 3m line with spacing of 75 cm between the sowing lines and in hills 30 cm apart. F₁s and parents were sown alternately (1hybrid / 1parent). Thinning was carried out at 2 plants per hill. Phosphate of ammonia (NPK 18-46-0) was provided at the dose of 100kg ^{-ha} or 22.5g / line. This supply was made 15 days after sowing. Urea was supplied at the rate of 50 kg ^{-ha} or 11.5g / line at first weeding. The tractor was used for the preparation of the soil (weeding), the plow for ridging. Weeding was done manually. The evaluation of cytoplasmic reaction was conducted as Three Female B-lines (recurrent parents) from Mali were crossed with the donor parent P851171, a line rich in proteins

(lysine) from Purdue University (USA). During the rainy season 2018, a total of 200 BC₁F₂ lines per population were planted for evaluation. Based on visual observations using the following parameters: grain quality, panicle yield and panicle shape selections were made. Thirty (30) panicles were selected per population, a total of 90. The 90 panicles selected were threshed and then sown by panicle (one seed), in the off season 2018-2019 to produce F₁ hybrids for the evaluation of the cytoplasmic reaction (test cross). The progenies selected were crossed with the female parent 12A (Source of cytoplasm A1). 75 F₁ obtained along with their recurrent parent were sown in the test cross trial during rainy season 2019. The sterile hybrid F₁ panicles identified were crossed with their parents BC₁F₄ in order to anticipate the conversion of the lines (BC₁). Three panicles were bagged by self pollinisation bags in each elementary plot. Two crosses were made per elementary plot (A/B line).

Table 1: Plant material (BC₁F₃) for each population and their F₁ hybrids

Population	Number of progenies (BC ₁ F ₃) /Pop	Number of hybrids F ₁ obtained /Pop
216-2BCTP1 // P851171	30	29
150B // P851171	30	23
12B // P851171).	30	23
Total	90	75

Data collection

For 50% flowering time (Half) of the panicles in the plot were bagged with selfing bags preceding anthesis. Following 3 to 6 days, bags were taken off and each blossoming panicle was checked for the presence of a cloud yellow pollen grains reflecting the fertility of the plant. The absence of the pollen indicated the sterility of the plant. For

anther morphology the method consists of looking at cautiously the anther form and color. The plants with whitish, thin, and scaly anthers were considered as sterile plants and those with plump and yellowish anthers were male- fertile. At physiological maturity, one panicle per plot (F₁ hybrids) was evaluated in relation to grain filling. Scale from 1 (0 seed) to 3 (100% seed set).

The process utilized for assessing these test crosses were those utilized by Reddy (1997) which are: Plants of the F₁ hybrids that displayed definitely no seed-set on all the selfing bag panicles addendum were considered as male sterile. The parents utilized as pollinators are named non-restorers and they can be utilized as a wellspring of new A-lines. Plants with complete seed-set on totally selfing bag panicles were classified as fertile. The relating pollen parents were named likely restorer or R-lines. They are to be utilized as male parents for hybrid production. Plants with a partial seed-set on totally bag panicles or those with a full seed-set on some selfing bag panicles and no seed-set in others, the relating male parents were rejected as they serve neither as restorers nor as maintainers. The entries and checks of the data collected were carried out with the Excel version 2017 spreadsheet as well as the yield of plot.

Conversion of identified B-lines containing high lysine and threonine to A-lines.

Based on the results of amino acids and cytoplasm reaction, 9 B line containing high lysine and threonine and theirs respective F₁ (A) were selected for this activity (Table 2).

The evaluation of the cytoplasmic reaction (Test cross) during rainy season 2019 permitted to

identify a number of B lines which were threshed and then sown by line according to A / B line in the off-season 2019-2020 to continue the conversion of lines (BC₂) and to produce seeds for (BC₃). Based on visual and biochemical observations such as the following parameters: seed set, anther morphology and biochemical analysis of the grains, a total of nine (9) B lines were selected from all populations. These B lines were threshed then sown in the rainy season 2020 to continue the conversion and to produce seeds for (BC₄). Nine males sterile F₁ (A lines) and nine selected B lines were sown side by side in a simple plot for each A / B lines in order to facilitate backcrossing. Self-pollination bags were used for both parents before flowering to avoid cross-pollination. Bagged panicles contained the first date on which the first panicles appeared. The folded corners of the bag are stapled. The 50% flowering date takes place between three and six days. All bagged panicles should be developed so that only marked bags should be inspected for flowering panicles. The pollen collection procedure determines the number of seed delivered by each cross. The pollen source panicles were collected early in the morning and kept in their self-pollinisation bags. The maintainers' panicles (B lines) were bagged and marked (year, location, type of test, plot number and date of pollen collection).

Table 2: List of B-lines containing high lysine and threonine

Sr. No.	Source	Pedigree	Content in mg/100g of grain	
			Lysine	Threonine
1	BC1F3-6	216-2BCTP1//P851171	10.79	13.13
2	BC1F3-7	216-2BCTP1//P851171	11.56	15.66
3	BC1F3- 8	216-2BCTP1//P851171	13.39	16.99
4	BC1F3-14	216-2BCTP1//P851171	0.17	0.06
5	BC1F3-20	216-2BCTP1//P851171	12.32	14.86
6	BC1F3-6	150B//P851171	8.59	9.86
7	BC1F3-16	150B//P851172	14.31	20.81
8	BC1F3-19	150B//P851173	9.14	11.41
9	BC1F3-13	12B//P851171	8.43	10.08

Panicles were identified and counted to be ready to receive pollen. The self-pollinisation bags were removed from the B lines then its pollen was poured on the corresponding F₁ (A lines) panicle without loss of pollen. The bottom of the bags was tightened with one hand around the upright peduncle with the other and vigorously shaken up and down so that its internal atmosphere is empty on the F₁ (A lines). The F₁ (A lines) panicles were again bagged and marked (year, location, type of test, plot number, date of pollen collection and name of female parent). Harvest the backcrossed A-line panicles and selfed pollinators' panicles individually and pair them as per the pollination done. Three A/B lines were harvested for each parent in each backcrossing generation. The use of caches helped flower induction of photoperiod-sensitive lines during the conversion of identified B-lines to A-lines and then accelerating the backcrossing generations.

Data collection was made on the following parameters

For Panicle examination before and after anthesis panicle examination before and after anthesis were made by taking images in different stage of panicles development. For grain quality (GQ), it is the visual appreciation of assemble of traits including the shape of the seed, the size, the presence or absence of mold or testa on the

grains. The scale of 1 (rejection), 2 (bad), 3 (passable), 4 (good) and 5 (excellent) was used for the rating. For Endosperm texture (ENDOS) it is a visual appreciation after having broken the seed into two parts. Data were taken according to the following scores: 1(Completely corneous; 2 (Mostly corneous); 5 (Intermediate); 7(Mostly starchy) and 9 (Completely starchy). The entries and checks of the data collected were carried out with the Excel version 2013 spreadsheet.

Results and discussion

Cytoplasmic reaction of female parents (BC₁F₃)

Based on seed set and anther morphology under all bagged panicles, twenty-six (26) lines were shown to be maintainer of sterility (B lines) (Table 3). These can be used as a female parent in the development of hybrids. However, the color and form of anthers were white and thin. Sixteen lines had less 50% of grain, which can be classified as a partial maintainer of sterility or restorers of fertility (BR lines). The color and form of anthers were plump and yellowish (Table 3). Thirty-three lines were shown to be restorer of fertility (R lines). These can be utilized as a male parent in development of hybrids. The color and form of anthers were plump and yellowish (Table 3).

Table 1 :List of lines identified in testcross

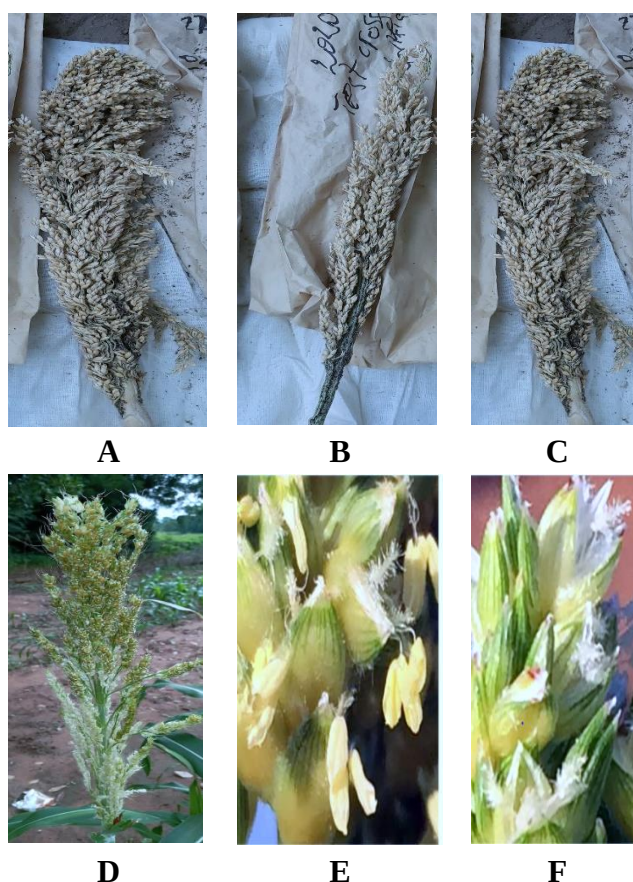
Score	Seed set	Number of hybrids	Anther Morphology	Type of line
1	(0 % grains)	26	White, thin	Maintainer of sterility (B)
2	(< 50 % grains)	16	Plump, yellow	Partial (BR)
3	(> 50 %)	33	Plump, yellow	Restorer of fertility (R)

These outcomes meet the prerequisites of Reddy *et al.*, (2009) that request that a male sterile line utilized for an enormous scope of hybrids should neither shed pollen, nor set seed when selfed, paying little heed to area or season. The presence of A new lines with high content lysine and threonine in the breeding program of Mali will improve sorghum production through high yielding hybrids development and high content protein. Notwithstanding the crosses that were sterile, sixteen crosses were discovered to be partial-sterile with the female 12A. These crosses were completely disposed from the test. Miller and Pickett (1964); Reddy (1997) recorded a few crosses, as partial-sterile. Male parents that are partial-sterile crosses were dismissed from a breeding system since they serve neither as restorers nor as maintainers reported by Reddy *et al.*, (2009). The numbers restorer lines were higher may be due to CMS steadiness comparable to temperature which is a significant factor in the production of hybrid seeds. The temperature stimulus in the length of flower development causes phenotypic variance for sterility in A1 male sterile lines According to Tarumoto *et al.*, (2008). At times, during the flowering time when crosses were assessed for sterility/fertility reactions, there was a risk of contamination which may have influenced the crosses. The contamination may be ascribed to the way that covered heads of hybrids were opened for confirmation of the presence of pollen and simultaneously for the assessment of the anther morphology. This may have brought about the flowers getting pollen from different panicles shedding pollen at that point. This likely affected the efficiency of male sterility expression in the production of hybrids. New techniques ought to hence be found to facilitate sterility/fertility assessment and to make the procedure more proficient.

Conversion of identified B-lines containing high lysine and threonine to A-lines

Panicle examination before and after anthesis compared with the normal female, the fortified females showed seed set with manual pollination at 50% and 100% anthesis (Figure 1 A and C). As shown Figure 1B, no seed set when the panicles of F₁ A were bagged with self-pollinated bags. The organs development shown by Figure 1 D, E and F, we observed the yellow anthers in B line at anthesis stage then white anthers in A line at anthesis stage. The study on the development of new female parents containing high content proteins (lysine and threonine) showed significant variability between lines. Similar results were obtained by Hulse and Pearson, 1980; Jambunathan *et al.*, 1984. The high lysine content of the lines could probably be and essentially due to the large quantity of glutelin, therefore rich in lysine, and the low fraction of prolamin can be explained by the absence of lysine. Similar results were found by Singh and Axtell (1973) and by Eggum *et al.*, (1990) then by Van Scoyoc, Ejeta, and Axtell (1988) obtained high lysine content in sorghum. These results showed that the lines developed had a high content lysine than the standards required by the FAO and Touré *et al.*, (2018). These new female lines are thus a source of energy not only for human food but also a good food for poultry, pigs and ruminants. The causes of malnutrition in Mali and Africa in general has shown that the most alarming nutritional problems are those of dietary protein and micronutrient deficiencies. Seed production of sorghum hybrids requires prior knowledge of the flowering date's behavior of two parents of hybrid (male parent and female parent). The study of the flowering date is of utmost importance in the process of producing hybrid sorghum seeds. Thus, Murty *et al.*, 1995 reported the importance of synchronization of the hybrid's parents. However, in our study on the development of new females containing high content lysine and threonine, we crossed the parents which synchronized in order to have a mastery of the flowering behavior of the B lines.

Figure 1 : Panicle examination before and after anthesis (A) Manuel pollinated showing normal seed set ; (B) Bagged panicle before anthesis; (C) Manuel pollinated at 50% of seed set, (D) Male sterility panicle; (E) Yellow anthers in Bline at anthesis stage and (F) White anthers in A line at anthesis stage



Morphological characterization of B lines

The grain qualities (GQ) varied from good (4) to excellent (5) for all the B lines. The endosperm (endos) textures also varied from intermediate starchy (5) to mostly starchy (Table 4). The grain weight BC1 to BC4 varied from 101 to 375 g for all the nine A lines (Table 4) and the plant height varied from 140 to 180 cm.

Females' parents had a height relatively smaller than Males' parents. Indeed, ideally for producing hybrid seeds it is preferable that the male parent is at least slightly higher than the female parent since the pollen which is dispersed with the wind has a downward movement. There would be no way to produce hybrid seeds by wind-linked pollination when the female is taller than the male as reported by Murty UR. (1999).

Table 4: Agronomical characteristics of selected lines

Sr. No.	Source	Pedigree	Grain quality	Endosperm textures	BC ₁	BC ₂	BC ₃	BC ₄
			From BC ₁ To BC ₄		Grain weight/cross (g)			
1	BC1F3-6	216-2BCTP1//P851171	4	7	250	300	315	375
2	BC1F3-7	216-2BCTP1//P851171	5	6	150	205	235	215
3	BC1F3-8	216-2BCTP1//P851171	4	6	311	312	312	313
4	BC1F3-14	216-2BCTP1//P851171	5	7	205	175	145	205
5	BC1F3-20	216-2BCTP1//P851171	5	7	145	165	165	201
6	BC1F3-6	150B//P851171	4	5	105	101	120	121
7	BC1F3-16	150B//P851172	5	6	203	185	154	250
8	BC1F3-19	150B//P851173	4	7	301	295	175	125
9	BC1F3-13	12B//P851171	4	6	101	108	201	215

BC : Backcrossing 1 to 4

Conclusion

Satisfactory results have been achieved through development of female parent lines with high lysine and threonine content. In the frame of test, the cytoplasmic restoration reaction of F₁ populations derived from backcrossing (BC₁F₃) with cytoplasmic male sterility. However, twenty-six lines were maintainer of sterility (B lines) with anthers color (white) and form (thin). Sixteen lines as partial maintainer of sterility or restorers of fertility (BR lines). The color and form of anthers (BR lines) were plump and yellowish. Thirty-three lines were restorer of fertility (R lines). The color and form of anthers (R lines) were plump and yellowish. Concerning the evaluation of female parents and panicle examination before and after anthesis of B line (BC₄). The nine B lines had a good grain qualities and endosperm textures. The grain weight was 101 to 375 g for all nine B lines.

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Reference

1. Axtell, J.D., Van Scoyoc, S.W. Christensen, P.J. and Ejeta, G. 1979. Current status of protein quality improvement in grain sorghum. Seed protein improvement in cereals and grain legumes. IAEA, Vienna.: 357.
2. Eggum, B.D. 1990. Importance of sorghum as a food in Africa. Pp. 222-228 in G. Ejeta, E.T. Mertz, L. Rooney, R. Schaffert, and J. Yohe, eds., Sorghum Nutritional Quality: proceedings of an international conference held February 26-March 1, 1990. Purdue University, West Lafayette. Indiana
3. House LR. 1985. A guide to sorghum breeding, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics ,Patancheru. 2: 502 324.
4. Hulse, J.H., Laing, E.M., and Pearson, O.E. 1980. Sorghum and The Millets: Their composition and nutritive value. Academic Press, London. ISBN: 0123613507. 877-965.
5. Jambunathan, R., Singh, U. and Subramanian, V. 1984. Grain quality of sorghum, pearl millet, pigeonpea and chickpea. In K.T. Achaya, éd. Interfaces between agriculture nutrition and food science. Proceedings of a workshop, Patancheru, Inde, 10-12 November 1981: 4760.

6. Kumar, A. A., Reddy, B.V.S., Reddy, P. S. and Ramaiah, B. 2010. Development of male sterile lines in sorghum. International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, Andhra Pradesh, India.
7. Kuhlman, L.C., Pring, D.R., Rooney, W.L., and Tang H.V. 2006. Allelic frequency at the Rf₃ and Rf₄ Loci and the Genetics of A₃ cytoplasmic fertility restoration in converted sorghum lines. *Crop Sci.*, 46:1576–1580.
8. Maunder, A.B. and Pickett, R.C. 1959. The genetic inheritance of cytoplasmic-genetic male sterility in grain sorghum. *Agronom., J.*, 51:47-49.
9. Moran, J.L. and Rooney, W.L. 2003. Effect of cytoplasm on the agronomic performance of grain sorghum hybrids. *Crop Sci.*, 43:777–781.
10. Murty D.S., Tabo R., and Ajayi.O., 1995. Sorghum hybrid seed production and management. Information bulletin, ICRISAT. 41 : 67.
11. Murty UR., 1999. Production de semences hybrides de sorgho dans hétérosis et la production de semences hybrides dans les cultures agronomiques .Amarjit S Barsa, éd. Etats-Unis, L' Haworth Press, Inc.6: 119-148.
12. Reddy, B.V.S. 1997. Development, production, and maintenance of male sterile lines in sorghum. p. 22-27. In: Singh, Faujdar, K.N. Rai, Belum V.S. Reddy, and B. Diwakar (eds.), training manual on development of cultivars and seed production techniques in sorghum and pearl millet. ICRISAT, Patancheru, AP. India. 8:5370-5375.
13. Reddy, B.V.S., Ramesh, S. and Sanjana Reddy, P. 2004. Sorghum breeding research at ICRISAT – Goals, strategies, methods and accomplishments. *International Sorghum and Millets Newsletter* 45:5-12.
14. Reddy, B.V.S., Ramesh, S., Sanjana Reddy, P. and Ashok Kumar, A. 2009. Male-sterility inducing cytoplasmic effects on combining ability in sorghum [*Sorghum bicolor* (L.) Moench]. *Indian J. Genet.*, 69(3):199-204.
15. Reddy, B.V.S., Thakur, R.P., Ramesh S., Rao, V.P. and Reddy P.S. 2006. Effect of cytoplasmic male sterility systems on sorghum grain mold development. *Int. Sorghum and Millets Newslett.*, 47:16-20.
16. Rooney, L.W. and Serna-Saldivar, S.O. 2003. Sorghum. In: Encyclopedia of food sciences and nutrition. 2nd ed. (B. Caballero, LC Trugo, PM Finglas, eds.).
17. Singh, R., and Axtell, J. D. 1973. High lysine mutant gene (hi) that improves protein quality and biological value of grain sorghum. *Crop Sci.*, 13: 535-539.
18. Stephens, J.C. and Holland, R.F. 1954. Cytoplasmic male sterility for hybrid seed production. *Agronm. J.*, 56:46:20.
19. Tarumoto, I., Ishii (Adachi), E., Yanase, M. and Fujimori, M. 2008. The Phenotypic fluctuation factor for male sterility in A1 male-sterile lines of sorghum [*Sorghum bicolor* (L.) Moench] *Sci. Rep. Graduate School of Life and Environmental Science. Osaka Pref. Univ.*, 59:1-6.
20. Tefera, A. 2020. Variability and association studies among the sorghum genotypes for seed yield and yield related traits under rainfed condition of Ethiopia. *J. Genet. Genom. Plant Breed.* 4(1):9-19.
21. Toure, A., 2018. Développement des lignées de sorgho [*Sorghum bicolor* (L.) Moench] fortifiées, à haut potentiel de rendement, adaptées aux zones soudano-sahéliennes du Mali, Unpublished, Thèse de Doctorat pour l'obtention du titre de Docteur de l'Institut Supérieur de Formation et de Recherche Appliquée (ISFRA) de Bamako. : 89-102.
22. Van Scoyoc, S.W., Ejeta, G. and Axtell, J.D. 1988. Kernel characteristics and protein traction changes during seed development of high-lysine and normal sorghums. *Cereal Chem.*, 65: 75-80.