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Full Length Research Paper

Physical characteristics of colza seeds treated and coated with different filling materials

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The knowledge about the physical characteristics of seeds, whether coated or not, is essential for the design of machines and equipment. Therefore, the objective of this work was to determine the physical characteristics of colza (*Brassica napus* L.) seeds coated with bentonite, gypsum and kaolin, treated with fungicide (carboxin + thiram) and aqueous extract of black pepper (*Piper nigrum* L.). The untreated colza seeds were submitted to the coating process using bentonite, gypsum and kaolin as fillers, and as treatment products were used as fungicide (carboxin + thiram) and an aqueous extract of black pepper which were added to an aqueous solution of 30% PVA glue (cementing mixture). Then the physical characteristics were determined: diameter, number of times increased, porosity, angle of repose, resistance, classification in sieves and weight of one thousand seeds. The experiment was organized in a completely randomized design and arranged in a factorial scheme. The means, when necessary, were compared by the Scott-Knott test. The colza seeds coated with bentonite presented greater diameter, number of times increased, porosity, angle of repose and weight of a thousand seeds, followed by gypsum and kaolin. The treatment products had little influence on the physical characteristics of the coated seeds.

Key words: *Brassica napus* L., diameter, resistance, weight of one thousand seeds.

INTRODUCTION

The colza seed (*Brassica napus* L.), or its improved variety, the canola, (*B. napus* L. ssp. *oleifera*) is one of the main oilseeds in the world. It has been used as green fodder for animal feed, fertilizer for soil conditioning and raw material for oil extraction, which has been used in human food, lighting, industrial use and more recently for the production of biofuel (Mori et al., 2014). However, its seeds are small in size, making it difficult to use them in

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the mechanized planting, and if they are sown broadcast, there is a risk of wind drift.

According to Duran and Retamal (1989), small and irregular seeds hinder precision sowing, when under certain circumstances the producer uses higher amounts of seeds in the establishment of the production fields to obtain the desired final stand.

In order to solve this problem, specialists have suggested the study of techniques for seed coating, where inlaying/pelletizing is indicated because of the use of a dry, inert material of fine granulometry and a cementing material (adhesive). This treatment allows the seed to obtain a rounded form, increasing its size and facilitating its distribution, be it manual or mechanical (Mendonça et al., 2007).

Although the technique has been developed for several years, information about the composition of the materials used and the coverings preparation are little spread, since this technique remains inaccessible along the seed companies and the companies that process coated/ pelleted seeds (Silva et al., 2002). The materials used in the coating/pelletizing process, including those of covering, adhesives and finishing, have influence in the final rigidity of the seed, in the water absorption and in the gas exchange between the seed and the external environment to the seeds; and all these aspects affect directly the germination (Silva, 1997; Silva and Nakagawa, 1998).

Additionally, there is the possibility of nutrients incorporation, growth regulators and other agrochemicals (insecticides and fungicides) during the coating/pelletizing process, which may constitute improvements in the seed health and in the seedling establishment (Silva et al., 2002). The use of plants with bioactive potential, in the form of extracts, oils and powders, against various organisms has been increasingly encouraged. Several researchers such as Cardoso et al. (2005), Bomtempo (2007), Bong (2010), Abbasi et al. (2010) and Khan et al. (2010) state that the piperine, main compound found in the black pepper (*Piper nigrum* L.), has a recognized cytotoxic, anti-inflammatory, antipyretic, analgesic, antioxidant, antitumor, antifungal and bactericidal activity.

What was been exposed shows the importance of studying filling and cementing materials that are easy to obtain, as well as products that can be used as seed treatment. Thus, the objective of this work was to determine the diameter, number of times increased, porosity, angle of repose, weight of one thousand seeds and resistance of colza (*Brassica napus* L.) seeds coated with bentonite, gypsum and kaolin and treated with fungicide or aqueous extract of black pepper (*P. nigrum* L.).

MATERIALS AND METHODS

Location of the experiment

The experiment was realized at the Laboratory of Storage and Processing of Agricultural Products (LAPPA), in the Federal University of Campina Grande, Campus of Campina Grande, Paraíba, Brazil.

Acquisition of the seeds

The seeds were purchased at the local trade of the city of Campina Grande, Paraíba, Brazil. After acquisition, the seeds were taken to the Laboratory for cleaning and removal of impurities that accompanied the seeds.

Preparation of the plant extract

The black pepper fruits (*P. nigrum* L.) were acquired at the central fair of the city of Campina Grande, Paraíba, Brazil. The aqueous extract was obtained from the fruits powder, which were weighed, dampened with distilled water, and left for maceration for 72 h, at room temperature of 24.0 ± 4.0°C in the absence of light and shaken daily for five minutes. The amount of powder used corresponded to 20% of the water volume. Subsequently, the solutions were filtered on filter paper, and the extract stored in an amber glass container with a capacity of 0.5 L (Almeida et al., 2004).

Materials and seed coating process

Three filling materials were used: (1) bentonite, (2) gypsum and (3) kaolin. As cementing material the PVA glue was used at the percentage of 30% for each filling material. As treatment products it was used as the aqueous extract of black pepper (*Piper nigrum* L.) and the carboxin + thiram fungicide corresponding to 50% of the mixture of each product, using 20% of distilled water to compose 100% of the mixture. The seed coating process occurred by the alternated application of cementing material and filling material. This process was repeated until all material destined to the process has been fully utilized.

Physical characteristics of the coated and treated seeds

Seed diameter and number of times increased

To determine the diameter of the coated seeds it was used as digital pachymeter with precision of 0.01 mm, using four repetitions of 25 seeds for each treatment. The results were expressed in millimeters (mm). The number of times increased was calculated by the ratio of the diameter of the coated seed to the bare seed. For statistical analysis, the mean of each 25 seeds was considered as repetition.

Classification by size

For the classification of the coated seeds by size, four subsamples of about 50 g were used per treatment. Each subsample was subjected to analysis by overlapping screen sieves of 1.0, 1.5, 2.5 and 4.0 mm. The set of sieves was shaken for a minute. The sieved fractions, including the portion that passed through the smaller sieve, were weighed and the weights of the fractions were expressed as a percentage of the total weight.

Porosity

The porosity was determined by the direct method, in which it was

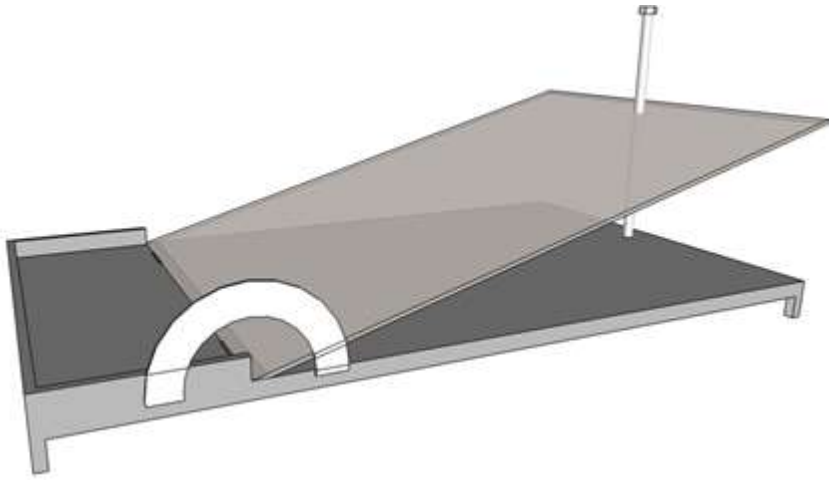

Figure 1. Equipment used to determine the angle of repose of colza (*Brassica napus* L.) seeds coated with bentonite, gypsum or kaolin and treated with fungicide or aqueous extract of black pepper (*Piper nigrum* L.).

obtained by adding a known volume of liquid sufficient to complement the empty spaces of the seed mass, using a burette and a measuring cylinder of 50 mL, in which one of them contained soybean oil and the other contained colza seeds coated with one of the combinations. As the oil was added to the measuring cylinder with seeds, it filled the empty spaces. By the difference between the amount of oil added to the measuring cylinder and the one contained in the burette with oil, the porosity of the coated seed mass was determined.

Angle of repose

In order to determine the angle of repose of the coated seeds it was used as an equipment made from a board with dimensions of 22 cm × 38 cm and an acrylic sheet with dimensions of 21 cm × 30 cm. These two pieces are joined by two hinges on one side. On the opposite side there is a screw that when it is screwed, lifts up the acrylic piece. On the same side of the hinges there is a protractor, located at the vertex formed between the board and the acrylic sheet. The moment when the seeds (2.0 g), which are located on the upper face of the acrylic sheet, move fully down, the angle in the protractor is measured (Figure 1).

Weight of one thousand seeds

To determine the weight of one thousand seeds, each combination of filling material and treatment product in thousand seeds were counted by replicate. After this, the seeds were weighed in a digital scale of precision and the data expressed in grams.

Resistance

To determine the percentage of resistant seeds, four repetitions of 100 colza seeds coated with bentonite, gypsum and kaolin, and treated with fungicide and aqueous extract of black pepper were used. These seeds were thrown at a 1.5 m height over a metal surface. This height was adopted because it is the average height in which the seeds are discharged in the seed tanks of the mechanized seeders. After that, the intact seeds out of the damaged ones were counted and the values transformed in percentage of the seeds that resisted the fall.

Experimental design and statistical analysis

The experiment was arranged in a completely randomized design. For the variables resistance and quantity of times increased, the factorial scheme 3 x 2 was used, as it did not require comparison with the control (bare seed) and for the other variables the factorial scheme 4 x 2 (filling materials x treatment products) was used. Each treatment was repeated four times. The data were submitted to Analysis of Variance ($P \leq 0.05$) and the means, when necessary, were compared by the Scott-Knott test ($P \leq 0.05$). For the values of the classification by size in the sieves, the means for each combination of filling material and treatment were presented, adding the standard error.

RESULTS

In Table 1, the mean squares values for seed diameter (SD), number of times increased (NTI), porosity (P), angle of repose (AR), weight of one thousand seeds (WTS) and resistance (R) of colza seeds coated with bentonite, gypsum and kaolin, and treated with fungicide (carboxin + thiram) and aqueous black pepper extract are organized. It was verified that there was an interaction effect between the factors for all the variables studied, except seed diameter (SD) and number of times increased (NTI). For the other variables there was an interaction effect between factors at 1 or 5% probability.

It is observed in Table 2 that there was no statistical difference between the treatment products within each filling material. There was also no statistical difference between the filling materials within each treatment product. On the other hand, for the means of the factor "filling materials" a statistical difference was observed

Table 1. Mean squares referring to seed diameter (SD), porosity (P), angle of repose (AR), weight of thousand seeds (WTS), number of times increased (NTI) and resistance (R) of colza seeds (*Brassica napus* L.) coated with different filling materials (FM) and treated with two treatment products (TP).

SV	DF	Mean Square				DF	Mean Square	
		SD	P	AR	WTS		NTI	R
FM	3	4.54**	522.03**	205.78**	424.98**	2	0.53**	12558.87**
TP	1	0.04ns	0.78ns	1.53ns	1.50*	1	0.02ns	222.04**
FM x TP	3	0.01ns	6.11**	1.95*	1.56**	2	0.01ns	260.04**
Error	24	0.01	0.718	0.41	0.84	27	0.004	4.43

(**) (*) (ns) Significant at 1, 5% and not significant, respectively.

Table 2. Means of the diameter (mm) of the colza seeds (*Brassica napus* L.) coated with bentonite, gypsum and kaolin, and treated with fungicide (carboxin+thiram) and aqueous extract of black pepper (*Piper nigrum* L.).

Filling materials	Treatment products		Means
	Fungicide	Plant extract	
Control	1.81 ± 0.005	1.81 ± 0.005	1.81[d]
Bentonite	3.58 ± 0.056	3.55 ± 0.055	3.57[a]
Gypsum	3.16 ± 0.029	3.10 ± 0.065	3.13[b]
Kaolin	2.74 ± 0.004	2.54 ± 0.061	2.64[c]
Means	2.83 [a]	2.75 [a]	2.81

*Means followed by the same lowercase letter in the column and row do not differ by the Scott-Knott test (P ≤ 0.05). CV% = 3.59.

Table 3. Means of the number of times increased of the colza (*Brassica napus* L.) seeds coated with bentonite, gypsum and kaolin, and treated with fungicide (carboxin+thiram) and aqueous extract of black pepper (*Piper nigrum* L.).

Filling materials	Treatment products		Means
	Fungicide	Plant extract	
Bentonite	1.98 ± 0.027	1.96 ± 0.031	1.97[a]
Gypsum	1.75 ± 0.016	1.71 ± 0.036	1.73[b]
Kaolin	1.51 ± 0.005	1.41 ± 0.034	1.46[c]
Means	1.75 [a]	1.70 [a]	1.73

*Means followed by the same lowercase letter in the column and row do not differ by the Scott-Knott test (P ≤ 0.05). CV% = 3.63.

between the treatments, with lower seed diameter recorded in the control (1.81 mm) and larger diameter when the seeds were coated with bentonite (3.57 mm). The other filling materials generated seeds with intermediate diameters, which were statistically different from each other (Table 2).

When comparing the treatment products within each filling material, as well as the filling materials within each treatment product, no statistical difference was observed (Table 3). However, for the means of the factor "filling materials", a statistical difference was observed, with the highest seed increase when using bentonite (1.97 times) and the lower when using kaolin (1.46 times). When gypsum was used, there was an increase of 1.73 times in relation to bare seed, which is an intermediate value, and statistically different from the other two filling materials.

It can be seen at Table 4 that the use of the treatment products had little influence on the seed classification. When the seeds were coated with bentonite and treated with fungicide or aqueous extract of black pepper, approximately 80% of the seeds were retained in the 1.5 and 2.5 mm sieves. Regarding the use of gypsum, approximately 65% of the seeds coated with this material were retained in the 1.5 mm screen sieve. The other

Table 4. Colza (*Brassica napus* L.) seeds coated with bentonite, gypsum and kaolin, and treated with fungicide (carboxin+thiram) or aqueous extract of black pepper (*Piper nigrum* L.), retained (%) in screen sieves of 1.0; 1.5; 2.5 and 4.0 mm.

Combinations	Classification (mm)			
	1.0	1.5	2.5	4.0
Control	100.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Bentonite + fungicide	15.6 ± 0.09	48.1 ± 0.01	34.0 ± 0.10	2.3 ± 0.17
Bentonite + plant extract	13.0 ± 0.28	55.5 ± 0.26	29.9 ± 0.08	1.6 ± 0.06
Gypsum + fungicide	11.4 ± 0.02	69.3 ± 0.22	14.0 ± 0.09	5.2 ±0.29
Gypsum + plant extract	11.7 ± 0.09	61.9 ± 0.10	14.3 ± 0.13	12.0 ± 0.12
Kaolin + fungicide	25.5 ± 0.19	69.0 ± 0.64	1.6 ± 0.30	3.9 ± 0.53
Kaolin + plant extract	29.3 ± 0.04	59.5 ± 0.04	7.1 ± 0.47	4.1 ± 0.41

Table 5. Means of the porosity (%) of the colza (*Brassica napus* L.) seeds coated with bentonite, gypsum and kaolin and treated with fungicide and aqueous extract of black pepper (*Piper nigrum* L.).

Filling materials	Treatment products	
	Fungicide	Plant extract
Control	36.00 ± 0.35^{dA}	36.00 ± 0.35^{dA}
Bentonite	52.75 ± 0.41^{aB}	55.25 ± 0.22^{aA}
Gypsum	51.50 ± 0.25^{bA}	52.00 ± 0.35^{bA}
Kaolin	46.00 ± 0.35^{cA}	44.25 ± 0.54^{cB}

*Means followed by the same lowercase letter in the column and uppercase in the row do not differ by the Scott-Knott test (P ≤ 0.05). CV% = 1.81.

sieves were responsible for retaining about 35% of the seeds. Similar to the gypsum, when the kaolin was used, most of the seeds were retained in the 1.5 mm sieve (approximately 60%). The sieve of 1.0 mm was responsible for retaining on average 30% of the seeds, and the 2.5 and 4.0 mm sieves retained approximately 10% of the seeds.

When comparing the treatment products within each filling material, it was observed for the control that the mean porosity was of 36%, and for the gypsum the porosity varied from 51.50 to 52.00%. For the bentonite, it was observed that the highest porosity was verified when using the aqueous extract of black pepper (55.25%) and the lowest when using the fungicide (52.75%), differing statistically from each other. Comparing the treatment products within the kaolin, it was verified that the highest porosity occurred when the fungicide was used (46%), differing statistically from the use of aqueous extract of black pepper (44.25%) (Table 5).

Comparing the filling materials within each treatment product it can be verified that for the two treatment products there was a statistical difference between the filling materials, with higher porosity values when using the bentonite (52.75-55.25%). On the other hand, the lowest values of porosity were verified in the control (36%). In relation to the seeds coated with gypsum, it was verified that they exhibited the second highest porosity among the materials, varying from 51.50 to 52.00%. Seeds coated with the kaolin had porosities varying from 44.25 to 46.00%, presenting intermediate values to the control and the gypsum (Table 5).

Comparing the treatment products within each filling material, it has been found that the control had a mean angle of repose of 16°. In relation to the bentonite there was no statistical difference, with angles of repose varying from 25.75 to 26.00°. In relation to the gypsum and the kaolin, the highest angles of repose were observed when using the aqueous extract of black pepper (22.75 and 16.25°, respectively), differing statistically from the angles of repose when using the fungicide (21.50 and 15.00º, respectively).

When comparing the filling materials within each treatment product, it is verified that the control had an angle of repose of 16°. For the fungicide, it was observed that the lowest angle of repose was observed when the kaolin was used (15.00°) and the highest when the bentonite (26.50°) was used. The other filling materials presented intermediate values for the angle of repose, varying from 16.00 to 21.50°. In relation to the aqueous extract of black pepper, it was verified that the lowest angle of repose was obtained when the kaolin was used (16.00°), being statistically equal to the control (16.00°).

Table 6. Means of the angle of repose (º) of the colza (*Brassica napus* L.) seeds coated with bentonite, gypsum and kaolin and treated with fungicide and aqueous extract of black pepper (*Piper nigrum* L.).

Filling materials	Treatment products	
	Fungicide	Plant extract
Control	16.00 ± 0.35 ^{cA}	16.00 ± 0.35 ^{cA}
Bentonite	26.50 ± 0.25 ^{aA}	25.75 ± 0.22 ^{aA}
Gypsum	21.50 ± 0.25 ^{bB}	22.75 ± 0.22 ^{bA}
Kaolin	15.00 ± 0.35 ^{dB}	16.25 ± 0.22 ^{cA}

*Means followed by the same lowercase letter in the column and uppercase in the row do not differ by the Scott-Knott test (P ≤ 0.05). CV% = 3.27.

Table 7. Means of the weight of one thousand seeds (g) of colza (*Brassica napus* L.) coated with bentonite, gypsum or kaolin and treated with fungicide or aqueous extract of black pepper (*Piper nigrum* L.).

Filling materials	Treatment products		Means
	Fungicide	Plant extract	
Control	3.603 ± 0.053	3.603 ± 0.053	3.603 ± 0.038 ^{d}
Bentonite	20.267 ± 0.545	20.693 ± 0.497	20.480 ± 0.377 ^{a}
Gypsum	15.211 ± 0.575	13.591 ± 0.374	14.401 ± 0.447 ^{b}
Kaolin	8.895 ± 0.400	8.357 ± 0.296	8.625 ± 0.266 ^{c}
Means	11.9938 ± 1.591 ^{a}	11.5605 ± 1.596 ^{a}	11.7771

*Means followed by the same lowercase letter in the column and uppercase in the row do not differ by the Scott-Knott test (P ≤ 0.05). CV% = 7.81.

Table 8. Percentage of colza seeds (*Brassica napus* L.) coated with bentonite, gypsum or kaolin and treated with fungicide or aqueous extract of black pepper (*Piper nigrum* L.) resistant to mechanical damage.

Filling materials	Treatment products	
	Fungicide	Plant extract
Bentonite	99.00 ± 0.50 ^{aA}	99.50 ± 0.43 ^{aA}
Gypsum	40.50 ± 1.64 ^{bA}	21.25 ± 1.29 ^{bB}
Kaolin	99.50 ± 0.43 ^{aA}	100.00 ± 0.00 ^{aA}

*Means followed by the same lowercase letter in the column and uppercase in the row do not differ by the Scott-Knott test (P ≤ 0.05). CV% = 2.75.

On the other hand, the highest angle of repose was observed when using the bentonite (25.75º) (Table 6).

In Table 7 show the mean values for the weight of one thousand colza seeds coated with bentonite, gypsum and kaolin and treated with fungicide and aqueous extract of black pepper. When comparing the treatment products within each filling material, as well as the filling materials within each treatment product, it was verified that there was no statistical difference, with the values of the weight of one thousand seeds varying from 3.603 (control) to 20.693 (bentonite). However, by observing the means of the filling materials factor, a statistical difference can be verified, with the lower weight of one thousand seeds being verified in the control (3.603 g) and the highest one when using the bentonite (20.480 g). The other filling materials presented intermediate weights ranging from 8.625 g (kaolin) to 14.401 g (gypsum).

When comparing the treatment products within each filling material, it is found for the bentonite and the kaolin that there was no statistical difference between the treatment products, with resistances varying from 99.0 to 100.0%. However, when the gypsum was used for coating the seeds, it was verified a higher resistance to the fungicide (40.50%), differing statistically from the use of the plant extract (21.25%) (Table 8).

By comparing the filling materials within each treatment

product in relation to the resistance, it was verified that for the two treatment products there was no statistical difference between the bentonite and the kaolin, with values varying from 99.0 to 99.50% for the bentonite and 99.50 to 100.0% for the kaolin. These two filling materials differed statistically from the gypsum, in which the resistance values were of 40.5 and 21.25% for the fungicide and the aqueous extract of black pepper, respectively (Table 8).

DISCUSSION

It was noticed that the filling materials provided changes in the physical characteristics of the colza seeds, especially the bentonite, which stood out over the other materials, presenting higher values for the seed diameter, increasing on average 200% of the original diameter of the seeds. The gypsum was also shown as a material capable of increasing the seed diameter; however, it increased the diameter by an average of 175%. In general, the bentonite generated seeds with larger diameter, followed by gypsum and kaolin.

In relation to the weight of one thousand seeds, all the materials made possible the increase in the weight of the seeds, however, the bentonite and the gypsum stood out for increasing approximately 550 and 400%, respectively, the weight of the seeds. The kaolin provided an average increase of 220%. This is something important because according to Miller and Sooter (1967), this increase in size and weight translates into seed economy, reducing or eliminating the thinning, thus presenting a uniform stand. While Borderon (1989) and Sachs et al. (1982) state that the increase in size and weight, enables precision sowing. Roos and Moore III (1975) confirm the previous observations, emphasizing the possibility of mechanized sowing because the technique not only standardizes size and weight but also shape.

In addition to the above, seeds coated with bentonite, regardless of the treatment product used, were mostly retained in the 1.5 and 2.5 mm screen sieves, which is approximately 80% of the seeds. As for the seeds that were coated with gypsum or kaolin, 60% of them were retained in the 1.5 mm screen sieve. Again, this shows the superiority of the bentonite as a filling material, being able to add larger amount of material on the surface of the seed.

In relation to the porosity, the bentonite was superior to the other materials, increasing in 52% the porosity in relation to the bare seeds. The gypsum and the kaolin increased by an average of 38 and 22%, respectively, the pore size. The knowledge of this physical characteristic is important, especially in silos, where larger seeds, consequently with greater porosity, allow greater efficiency of the aeration and drying processes, due to letting the air, whether warm or not, pass more efficiently through the seeds. In addition, it reflects on fan sizing, drying and aeration systems and engine power (Silva and Corrêa, 2000). Thus, seeds coated with bentonite would require less air flow and consequently less energy.

As for the angle of repose of the seeds, only the fillers bentonite and gypsum modified the angle of the seeds, having the bentonite increased an average of 10 degrees and the gypsum increased 5 degrees the angle of repose of the seeds. However, different angles of repose between the materials are probably due to the characteristics of the filling materials, which provide coated seeds with different finish levels, consequently with different roughness at the end of the process.

Regarding the resistance of the coated seeds, the bentonite and the kaolin presented high resistances (up to 100%) with any treatment product. However, the gypsum exhibited low resistances, being the highest when it was used together with the fungicide (approximately 40%). This characteristic, as well as its size, is important when working with coated or pelleted seeds because according to Silva and Nakagawa (1998), the resistance is related to the maintenance of the integrity of the coated seed during the processing, transportation and handling operations. According to these same authors, the difference between the materials is due to the physical characteristics of the materials themselves, which for bentonite and kaolin formed firm structures with a certain plasticity, not breaking up after the fall, whereas for the gypsum formed a rigid structure without plasticity, causing the break of the coating with the impact due to the fall of the seed.

In general, the bentonite was shown to be a viable material for use in the colza seeds coating process, as it increases them in size, weight and gives resistance to the coating; all desirable characteristics in a coated seed. According to Silva and Ferreira (2008) bentonite is employed in the industry as a binding agent and binder. These characteristics are probably responsible for differentiating the bentonite from the other materials when used in the incrustation of colza seeds. As a way of validating the bentonite as a material capable of coating seeds, germination and vigor tests are required, since this material may retard/inhibit the germination.

Conclusions

In view of the above, it can be concluded that: Colza seeds coated with bentonite present greater diameter, number of times increased, porosity, angle of repose and weight of one thousand seeds, followed by gypsum and kaolin. Seeds coated with bentonite and kaolin are more resistant to the fall, whereas the ones coated with gypsum are less resistant. Seeds coated with bentonite are retained, for the most part, in 1.5 and 2.5 mm screen sieves. The gypsum and the kaolin used in the coating provide seeds that are mostly retained in 1.5 mm screen sieves. The treatment products exert little influence on

the physical characteristics of the coated seeds. The combination of gypsum + fungicide increases the resistance of the coating.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abbasi BH (2010). Conventional and modern propagation techniques in *Piper nigrum*. Journal of Medicinal Plants Research 4(1): 7-12.

Almeida SA, Almeida FAC, Santos NR, Araújo MER, Rodrigues JP (2004). Atividade inseticida de extratos vegetais sobre *Callosobruchus maculatus* (Fabr., 1775) (Coleoptera: Bruchidae). Revista Brasileira de Agrociência 10(1):67-70.

Bomtempo M (2007). Pimenta e seus benefícios à saúde. São Paulo: Alaude.

Bong CFJ (2010). Pellitorine, a Potential Anti-Cancer Lead Compound against HL60 and MCT-7 Cell Lines and Microbial Transformation of Piperine from *Piper Nigrum*. Molecules 15(4):2398-2404.

Borderon MA (1989). Enrobage et pelliculage: La semence habillée. Cultivar 246:77-78.

Cardoso JFR, Evangelista DW, Viana EB, Lima MEF, Soares BA, Barreto Junior CB, Brito MF, Mazur C, Danelli MGM (2005). Avaliação do efeito tóxico da Piperina isolada da pimenta do reino (*Piper nigrum* L.) em camundongos. Revista Universidade Rural 25(1):85-91.

Duran JM, Retamal N (1989). Semillas "sintéticas" y biotecnología. II Symposium Nacional de Semillas, Sevilla.

Khan S, AnwarF, Abdin MZ (2010). Development of RAPD markers for authentication of *Piper nigrum* (L.). Environment *&* We: An International Journal of Science and Technology 5:47-56.

Mendonça EAF, Carvalho NM, Ramos NP (2007). Revestimento de sementes de milho superdoce (SH2)1. Revista Brasileira de Sementes 29(2):68-79.

Miller WF, Sooter C (1967). Improving emergence of pelleted vegetable seed. Transactions of the ASAE 10(5):658-666.

Mori C, Tomm GO, Ferreira PEP (2014). Aspectos econômicos e conjunturais da cultura da canola no mundo e no Brasil (Embrapa Trigo. Documentos, 149). Passo Fundo, Embrapa Trigo.

Roos EE, Moore III FD (1975). Effect of seed coating on performance of lettuce seeds in greenhouse soil tests. Journal of the American Society for Horticultural Science 100:573-576.

Sachs M, Cantlife DJ, Nell TA (1982). Germination behavior of sand-coated sweet pepper seed. Journal of the American Society for Horticultural Science 107:412-416.

Silva ARV, Ferreira HC (2008). Argilas bentoníticas: conceitos, estruturas, propriedades, usos industriais, reservas, produção e produtores/fornecedores nacionais e internacionais. Revista Eletrônica de Materiais e Processos 3(2):26-35.

Silva JS, Corrêa PC (2000). Estrutura, composição e propriedades dos grãos. In: Silva JS Secagem e Armazenamento de produtos agrícolas. Juiz de Fora: Instituto Maria pp. 21-37.

Silva FS, Corrêa PC, Calil-Júnior C, Gomes FC (2006). Ângulo de repouso, atrito interno e efetivo dos grãos de café com pergaminho. Revista Brasileira de Produtos Agroindustriais 8(1):17-23.

Silva JBC (1997). Avaliação de métodos e materiais para peletização de sementes. (doctoral dissertation). Faculdade de Ciências Agronômicas, Universidade Estadual Paulista, Botucatu, São Paulo, Brasil.

Silva JBC, Santos PEC, Nascimento WM (2002). Desempenho de sementes pelotizadas de alface em função do material cimentante e da temperatura de secagem dos peletes. Horticultura Brasileira 20(1):67-70.

Silva JBC, Nakagawa J (1998). Confecção e avaliação de péletes de sementes de alface. Horticultura Brasileira 16(2):151-158.

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Yield and water use efficiency of cauliflower under irrigation different levels in tropical climate

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The aim of the present study is to assess the effects of different irrigation blades on the growth and yield of cauliflower cv. Verona CMS. The plants were cultivated in Red-yellow Latosol during the dry period (winter-spring 2014 and 2015), in the Cerrado-Amazon transition region, Middle-Northern of Mato Grasso State, Brazil. The reference evapotranspiration (ETo) was recorded through the Class A pan method by using tank coefficient of (Kp) 0.7795. We assessed the blades of 40, 60, 80, 100 and 120% evapotranspiration of the culture (ETc) by taking into account the cultivation coefficients (Kc) of 0.70 and 0.95 in the vegetative and reproductive phases. With regard to the morphometric variables of the plants (height, stem diameter and number of leaves) there were no significant interactions between assessment time (days after planting) and irrigation blades throughout the crop years. The hydric response functions presented higher yield at repositions from 80% to 100% of ETc. Variations in the irrigation blade did not influence the thermal demands for the inflorescence differentiation period or the inflorescence shape. Increased irrigated blade reduced water use efficiency: 7.4 and 12.4 kg of fresh mass per m³ of irrigated water.

Key words: Irrigation management, water response function, degree-days, evapotranspiration.

INTRODUCTION

The space distribution of vegetable production centers in Brazil depends on economic, logistical, social and environmental factors (Garcia Filho et al., 2017). Mato Grosso State presents some obstacles for the offer of vegetables due to the high transportation costs and quality losses on products to be consumed *in-natura*,

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mainly in the North of the State. The fast economic growth in some counties (for instance, Sinop: 51.12 and 10.4% population and economic growth in the last decade – IBGE, 2010) led to fast growth of local demands for fruits and vegetables. However, along with the distance from producing centers, knowledge about the yield and physiological features of crops traditionally cultivated in temperate climate regions or in high altitude areas remains poor.

The vegetable production sector has great social importance because of its high demand for man-power and the generation of many direct and indirect job positions in all production stages, including trading. However, the sector faces high risks due to phytosanitary issues, its high sensitivity to weather conditions, its high vulnerability to offer and market seasonality, among others (Zanuzo et al., 2013; Garcia Filho et al., 2017; Ribeiro et al., 2017). The vegetable production sector demands faster development of new technologies than other production systems in order to comply with the different regional conditions in Brazil. Such need results from intrinsic peculiarities of product diversity, production cycle duration, input features and demands, irrigation, fertilization and culture traits.

The aforementioned scenario highlights that the horticultural sector can also have strong environmental impact caused by the use of agricultural pesticides, chemical fertilizers and by the capture of irrigation water. Therefore, the sector has been looking for sustainable management procedures capable of preserving the natural resources and of replacing the conventional production systems (Souza et al., 2013). The absence of appropriate water management is easily observed in irrigated areas, and it results in excessive water and energy use and waste, besides causing environment and plant phytosanitary issues. On the other hand, irrigation deficit impairs the development of culture because it reduces irrigation development and makes the final product unviable for trading and consumption. Therefore, such deficit results in reduced yield and, consequently, in losses to producers (Pereira et al., 2016; Domínguez et al., 2017; Lellis et al., 2017; Koksal et al., 2017; Seidel et al., 2017).

Thus, appropriate irrigation management practices help to increase yield, improve the quality of horticulture products, minimize water use and preserve the water resources, mainly in tropical climate regions facing water restriction periods. Irrigation management sets the time for irrigation and the amount of water based on culture demand, in order to get an efficient irrigation water management. It is essential to know the water needs of the culture in its different phenological phases, which are given by the evapotranspiration potential and water response functions (Sarkar et al., 2009; Souza et al., 2011a, b; Yavuz et al., 2015). Overall, vegetables are extremely dependent on appropriate water input, in all development phases, for biomass production at acceptable amounts and quality. This sector is one of the most demanding in the agricultural sector due to its irrigation water demand (Sousa et al., 2011; Tomassoni et al., 2013; Silva et al., 2014; Léllis et al., 2017; Seidel et al., 2017). Thus, the search for sustainable management ways capable of allowing the preservation of natural resources and the replacement of conventional production systems is growing (Souza et al., 2013).

Among the many vegetables available in Brazil is cauliflower (*Brassica oleracea* L. var botrytis L.) belonging to Brassicas family; it is included in the group of the most consumed ones, due to its high nutrition and commercial value (May et al., 2007; Torres et al., 2015; Garcia Filho et al., 2017). Light and temperature are among the environmental factors mostly limiting cauliflower cultivation in different times of the year and locations. Vegetables present reduced cultivation cycle when they are grown under temperature conditions above the proper rates recommended for their growth. Such feature reflects on their yield and quality (Puiatti and Finger, 2009) indicating that the weather conditions in some regions can limit their cauliflower production in a part of the year. The ideal environment or time of year, for cauliflower cultivation has been the main focus of countless research (Zanuzo et al., 2013; Ribeiro et al., 2017).

Cauliflower is sensitive to water deficit (Kochler et al., 2007; May et al., 2007; Tomassoni et al., 2013; Pereira et al., 2016). Its yield-response is influenced by irrigation amount and frequency, water application method, culture development stage, water physical and soil conditions, and micro-climatic conditions in the region (Sahin et al., 2009; Sarkar et al., 2009, 2010; Oliveira, 2015; Seidel et al., 2017).

Accordingly, it is essential to know the water needs of this culture and its water response functions in order to achieve efficient irrigation water management (Souza et al., 2011a). Thus, the aim of the present study is to assess the agronomic development of cauliflower cv. Verona CMS under different irrigations blades in Middle-Northern Mato Grosso State, Brazil.

MATERIALS AND METHODS

Study site

The experiment was conducted in the Vegetal Production Sector of UFMT, Sinop Campus (11.85°S and 55.38° W, altitude 371 m). Plants were cultivated in dystrophic Red-yellow Latosol (EMBRAPA, 2013) in two different production cycles at the transition between the dry and the rainy season (from June to November) in 2014 and 2015. The climate in the region was of the Aw type according to the Köppen classification (tropical warm and humid), with two well-defined seasons: rainy (from October to April) and dry (from May to September). These seasons, in their turn, have straight influence on solar radiation transmissivity (Souza et al., 2016) and on other meteorological elements (Table 1). The mean annual potential rainfall and evapotranspiration recorded 1974.77 and 1327.29 mm, respectively, and the mean monthly temperature varied from

Table 1. Mean monthly behavior of the meteorological variables in Middle-Northern Mato Grosso State from 06/1972 to 06/2010.

Months	Rainfall (mm)	Insolation (hours)	Maximum temp. (°C)	Average temp. (°C)	Minimum temp. (°C)	Relative humidity (%)	Wind speed ($m\ s^{-1}$)	Cloudiness time (hours)	Atmospheric pressure (hPa)
January	310.85	124.98	31.53	24.89	20.74	85.54	1.31	5.45	967.09
February	348.39	117.77	31.62	24.90	20.61	85.34	1.27	5.43	968.56
March	288.19	138.50	32.09	25.13	20.68	84.37	1.20	5.24	967.67
April	120.75	181.79	32.68	25.31	20.27	81.39	1.23	4.56	967.87
May	25.90	224.62	32.41	24.49	18.57	77.01	1.34	3.86	969.70
June	7.99	248.88	32.62	23.20	15.99	72.68	1.41	2.48	970.65
July	4.88	263.22	33.06	22.96	15.26	67.98	1.45	2.01	971.66
August	9.50	229.44	34.43	24.12	16.42	65.99	1.43	2.30	969.86
September	60.21	154.08	34.35	25.43	18.90	71.18	1.31	3.80	968.71
October	182.23	160.98	33.47	25.76	20.52	78.31	1.39	5.16	967.68
November	271.04	128.58	32.30	25.28	20.64	83.45	1.30	5.50	966.60
December	344.54	120.09	31.46	24.93	20.69	85.04	1.32	5.50	966.64

The conventional meteorological station belonged to the National Meteorology Institute (INMET), the so-called “Gleba Celeste”, located at 12.29° S and 55.29° W, altitude 415.0m.

23.2 to 28.8°C (Souza et al., 2013). Meteorological data were collected by an automatic station through the CR 1000 data acquisition system during the experimental period, and through global solar radiation (piranometer CS300), wind speed and direction (anemometer, 03002-L RM YOUNG) sensors, as well as through a psychrometer with thermometer shelter (CS 215) and a rain sensor (TE 525).

Experimental design

The two experiments were conducted in the same site; however, millet (*Pennisetum americanum* L. cv. ANm 17) was pre-cultivated (further cutting, fragmentation and incorporation to the soil through harrowing) in order to improve the amount of organic matter in the rainy season of the 2015 crop. A completely randomized block design, with four repetitions (20 useful plants) and 5 daily irrigation-blade treatments were adopted. The drip irrigation system with self-compensating emitters, at flow rate of 7.0 $L\ h^{-1}\ m^{-1}$ and service pressure of 10mca was employed.

The irrigation blades in 2014 (from July to November) were 60, 80, 100 and 120% of the evapotranspiration expected for the culture (Tec); in 2015, the blade was adjusted to 40% of ETC. The cultivation coefficients (kc) of 0.70 and 0.95 were used in the two production cycles in the vegetative and reproductive phases, respectively (Allen et al., 1998). The reference evapotranspiration (ETo) was recorded through the Class A tank method by taking into consideration the correction coefficients (kp) described by Souza et al. (2015) and Pedrosa et al. (2017).

$$ETo = ECA * Kp \quad (1)$$

$$ETc = ETo * Kc \quad (2)$$

where: ET_0 is the daily reference evapotranspiration ($L\ m^{-2}$); ETc is the evapotranspiration of daily culture ($L\ m^{-2}$); ECA is the evaporation of the daily class A pan ($L\ m^{2}$); Kp is the pan coefficient; Kc is the crop coefficient, which depends on the development stage.

Agronomic practices

Verona CMS (the assessed hybrid) has a good commercial acceptance and is recommended for cultivation in summer, since it is resistant to black rot. It has a white color inflorescence from 1.2 to 1.5 kg, besides presenting an approximate 100-day cycle (May et al., 2007; Zanuzzo et al., 2013;). The seedlings were produced in trays (128 cells) under 50%-shade black polyester fabric and capillary root zone irrigation. The transplantations were performed when plants presented between 4 and 5 expanded leaves (at August 1st, 2014 and July 11th, 2015) at spacing of 1.0 × 0.6 m (between rows and between plants). The soil in the experimental site presented 340, 170 and 495, 300, 188 and 512 g dm^{-3} of sand, silt and clay, at layers of 0-20 and 20-40 cm down the soil, respectively. The observed chemical features in the two cultivation years are shown in Table 2. In addition, values of 30.51 and 42.84 g dm^{-3} of total organic matter in the soil were found at 0-20 and 20-40 cm layers, respectively.

Cultivations were carried out under full sun light, without using dead cover on soil surface. Based on Filgueira (2005), 80, 350 and 200 kg ha^{-1} of N, P_2O_5 and K_2O, respectively were applied in the transplantation pit (base fertilization). Next, complementation with 150 kg ha^{-1} of N was conducted at 20, 40 and 60 days after transplantation (DAT). The application of 2.4 kg ha^{-1} of boron (borax – at 11% boron concentration) was performed on soil surface, close to the root system. Spontaneous vegetation control (weeds) was conducted through mechanical fashion and constant manual weeding in order to minimize competition with the culture. The phytosanitary treatments were performed whenever necessary, based on Figueira (2005) and May et al. (2007).

Crop observations

Non-destructive fortnight evaluations were conducted up to 120 DAT as a way to set plant height, stem diameter and number of leaves. The equatorial and longitudinal diameters, leaf area, fresh mass at inflorescence and shoot dry mass were obtained during the harvest (Hortibrasil, 2018). The yield performance was assessed through water use efficiency, by taking into account the water

Table 2. Chemical features of the soil in the experimental site at the two assessed depths in two production cycles.

Year	Depht of soil (cm)	pH (H_2O)	P (mg/dm³)	K	Ca	Mg	Al	H	S	T	V (%)
				cmol/dm³							
2014	0-20	5.7	2.44	0.14	1.9	1.16	0	3.1	3.2	6.3	50.78
	20-40	5.4	3.85	0.31	2.19	0.78	0.15	4.85	3.28	8.28	39.6
2015	0-20	6.1	2.09	0.06	1.97	0.78	0	3.63	2.81	6.44	43.63
	20-40	5.5	1.18	0.05	0.62	0.31	0.1	4.03	0.99	5.12	19.27

S: sum of the bases; T: CTC at pH 7.0; V: saturation of the bases (%).

volume of the irrigation blade, the rainfall records in inflorescence fresh mass production (EUA^1) and the water volume applied through irrigation (EUA^2). The meteorological measurements were collected every 5 min and stored, based on hourly and daily scales. The minimum (T_b) and maximum (TB) basal temperatures of 14 and 36°C (Nowbuth and Pearson, 1998) were applied in order to find the thermal sums (accumulated degree-days) for cauliflower plants. The proposal by Ometto (1981) was applied to cases suitable for it which depends on the local weather conditions, according to Souza et al. (2011b).

i) Case 1: Tm > Tb; TB > TM

$$GDD = ((TM - TB)^2)/2 + (Tm - Tb) \quad (3)$$

ii) Case 2: Tm ≤ Tb < TM ; TB > TM

$$GDD = ((TM - TB)^2)/(2(TM-Tm)) \quad (4)$$

iii) Case 3: Tb < Tm; TB < TM

$$GDD = 2[(TM\ Tm)\ (Tm - Tb)] + ((TM - Tm)^2) - ((TM - TB)^2) / (2(TM - Tm)) \quad (5)$$

iv) Case 4: Tb > Tm; TB < TM

$$GDD = 0.5\ [((TM - Tb)^2) - ((TM - TB)^2)] / (TM - Tm) \quad (6)$$

Where: TM and Tm = maximum and minimum daily temperatures (°C). Tb and TB = minimum and maximum basal temperature

Statistical analysis

Data were subjected to analysis of variance; means were compared through the Tukey test at 5% probability whenever they were significant. Regressions were adjusted by taking into consideration the irrigated blade as independent variable. The statistical package Sisvar 5.5 Build 82 was used in the calculations.

RESULTS AND DISCUSSION

Meteorological dates

The climatic seasonality in the Sinop-MT region (Table 1) showed that the time to conduct the experiment was appropriate to assess the effect of irrigation blades, regardless of the year. Accumulated rainfall recorded 424.6 and 88.0 mm in 2014 and 2015, respectively. However, in 2014 only 50 mm was recorded up to 80 DAT (10/19th/14) (Figure 1). Such behavior was essential to minimize soil humidity homogenization (due to rainfall), mainly during culture vegetative development, which, in turn, would have direct influence on the hydric deficit effect over the productive components.

According to Santos et al. (2013), there are wider thermal amplitudes in winter in Sinop-MT region, since water steam has great potential to mitigate radiation in the atmosphere. Thus, the differences between night and daylight temperature in the rainy months was caused by radiation transmissivity reduction (daylight cycle) and by radiation incidence increase in long waves emitted by the atmosphere (night cycle). There was mitigation of the maximum temperature due to changes in the total of direct and diffuse components, caused by cloudiness and minimum temperature increase. This outcome resulted from balance of night long-waves (Souza et al., 2016). Accordingly, they recorded daily mean temperatures of 26.38, 27.32, 27.27 and 25.88°C between August and November 2014, and 25.17, 26.49, 28.38, 27.92 and 26.52°C between July and November, 2015, respectively.

Another significant effect of rainfall could be observed on the global radiation incidence (short waves) between the 2 production cycles. In 2014, the daily means were 27.16, 19.82, 20.53 and 18.32 MJ m^{-2} day^{-1} between August and November, whereas in 2015 (lower accumulated rainfall throughout the cultivation cycle), the daily means were 21.15, 22.34, 21.35, 21.88 and 18.63 MJ m^{-2} day^{-1} between July and November, respectively. If one takes into consideration that global radiation is formed by visible and infrared ultra-violet radiation, it was possible indicating more availability of photosynthetically active radiation in the winter of 2015, fact that led to better yield performance at the same water-reposition level. The daily thermal amplitudes varied from 14.7 to 20.0°C in August and from 4.94 to 14.4°C in November, 2014, whereas, in 2015, they varied from 15.1 to 21.5°C in July and from 7.9 to 14.0°C in November.

Cauliflowers grown under Brazilian conditions were recommended for typically temperate climate; however, nowadays it has been gaining space in tropical climate regions. The difficulty in producing winter cultivars in hot weather regions was based on the need of mild temperatures (14-20°C) to pass from the vegetative to the reproductive stage (Filgueira, 2005). Genetic enhancement made it possible to select cultivars capable

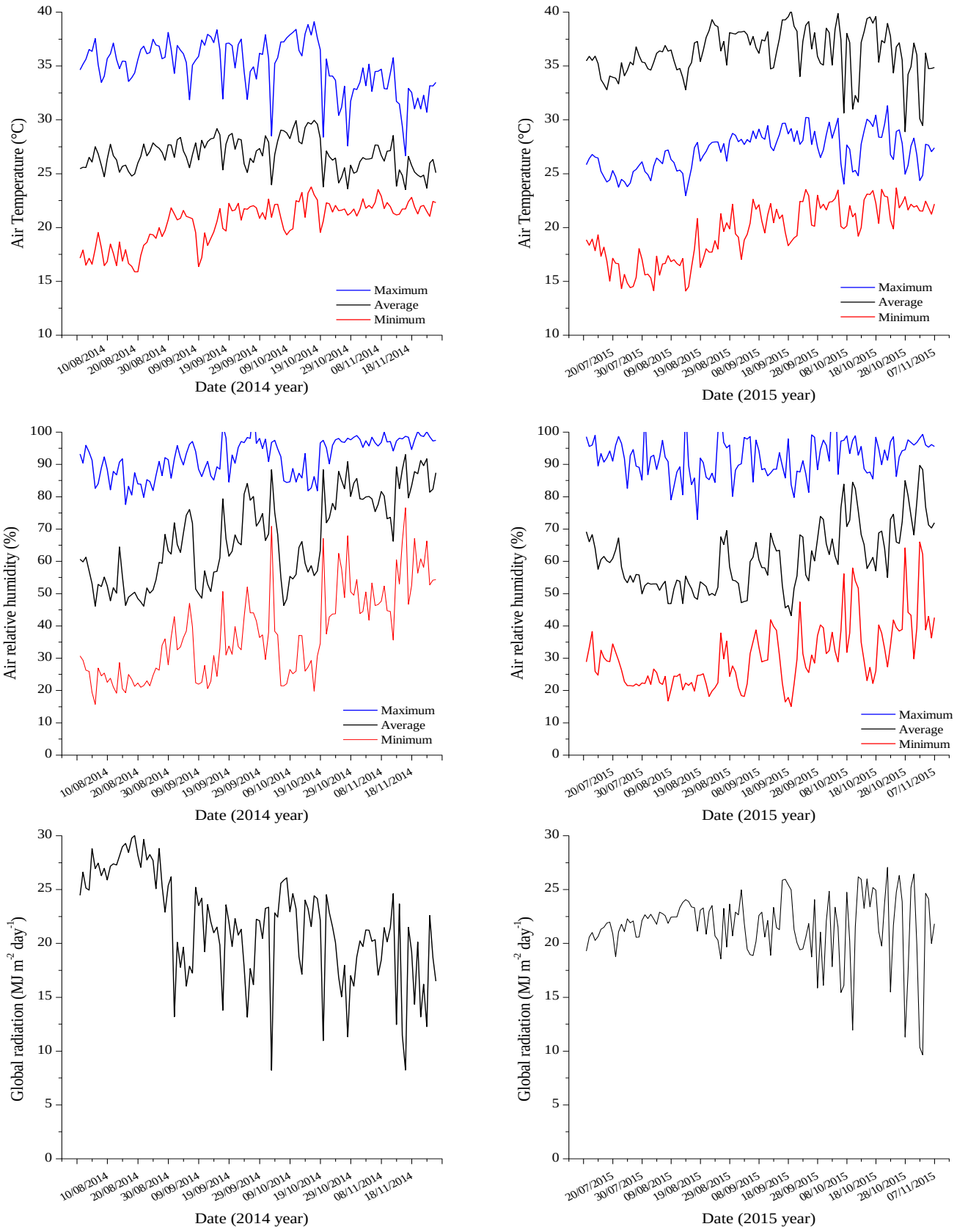

Figure 1. Air temperature variations, relative air humidity, global radiation, rainfall and reference evapotranspiration in Sinop-MT, from August to November, 2014 and 2015.

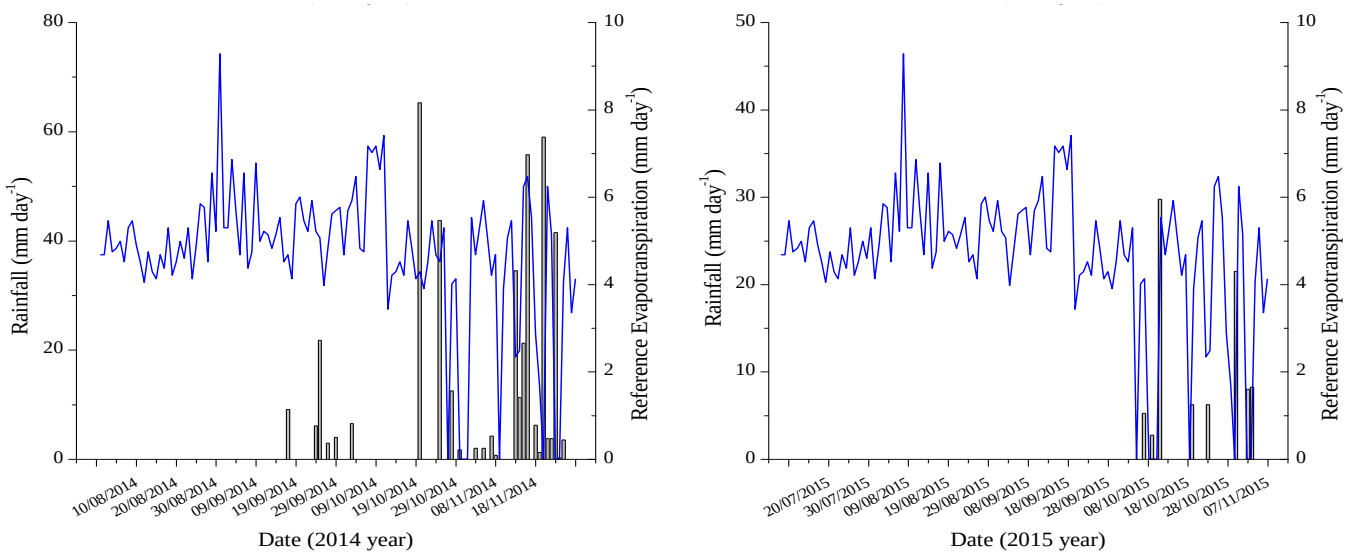

Figure 1. Contd.

of tolerating temperatures above 30°C (May et al., 2007) and it enabled cultivating this vegetable in tropical climate regions, as long as cultivars presenting wide adaptability were selected. Such process allowed these cultivars to be grown all year long (Claudio, 2013).

According to Filgueira (2005), there are cultivars with different thermo-climatic demands in Brazil, which can be gathered in two groups: the first one comprises cultivars adapted to plantation in fall-winter and the second one covers spring-summer cultivars, which do not demand cold, as well as develop and produce under higher temperatures. There are few open pollination cultivars in the second group, and the current trend is to replace them by hybrids (Cardoso and Silva, 2009). Zanuzo et al. (2013) assessed 8 genetic materials from different thermo-climatic demands in Sinop-Mt region. They concluded that hybrids belonging to group Verona present wide adaptability to regional conditions. Accordingly, despite the wide thermal amplitude in winter, due to rainfall, there was maximal temperature reduction and minimal temperature increase, so that temperatures within the ideal rate for the cultivation of hybrids belonging to group Verona were reached. These species were recommended for summer cultivation (Morais Junior et al., 2012; Zanuzo et al., 2013).

Table 3 synthesizes information encompassed in the monthly scale, total rainfall, evapotranspiration and the blade applied throughout the 117 (1149 degrees-days accumulated - GDA) and 119 (1396.4 GDA) cultivation days in 2014 and 2015. Such periods-of-time corresponded to the accumulation of 1449 and 1396.4 degrees-day, respectively. In Table 2, the effective rained blade was only higher than ETo in October and November 2014. Therefore, there was need of water reposition for 277.62 and 282 mm irrigation throughout the productive cycles. These values were references for the treatment with blade reposition of 100% ETc.

Rainfall changed the applied blade in the treatments because the experiment was conducted under field conditions. Thus, after irrigation (I) was added to the effective rainfall (Ef Ra), it was possible to verify the blades corresponding to 84.92 and 108% of ETc applied to treatments T1, T2 and T4 in 2014; to 54, 70, 85 and 115% of ETc to treatments T1, T2, T3 and T5 in 2015. However, all analyses linked to water use efficiency were taken into account, based on the initial % of the applied blade, since the differences between the two cultivation cycles were lower than 5.0mm when the treatments were defined.

The analysis of variance (ANOVA) did not show significant interactions between evaluation time (DAT) and the irrigation blade, regardless of the crop year. Isolated factors presented significant differences, and the effects throughout time (DAT) were assessed through regression based on the thermal sum (GDA) (Figure 2). The irrigation blades influenced plant height and the number of leaves from the 103 DAT and after on, in the 2014 production cycle. However, plants presented from 22.4 to 28.6 leaves between 89 h and 117 DAT (Table 4). The decreased number of leaves recorded at the end of the cycles resulted from the senescence of leaves closer to the soil (first leaves emitted by the plants).

On the other hand, the irrigation blades had an influence after the 109 DAT in the 2014 cycle. They led to higher mean height, wider stem diameters and larger number of leaves in the blades of 60 and 100% ETc reposition treatments. There was reduced growth and plant height in this production cycle, regardless of the

Table 3. Total mean values of effective rainfall (Ef Ra), reference evapotranspiration (ETo) and total blade applied through irrigation + effective rainfall (ETreal) during the evaluation periods, with treatment using 100% ETc reposition.

Year	Mês	Efective rainfall (Ef Ra) (mm)	Reference evapotranspiration (mm)	Applied wather depth (mm)	ETreal (mm)
2014	July	-	-	-	-
	August	0	161.2	80.6	80.6
	September	43.9	128.2	73.7	117.7
	October	129.7	119.6	83.9	213.6
	November	251.0	56.4	39.5	290.4
	Total	424.6	465.4	277.7	702.3
2015	July	0	99.4	49.7	49.7
	August	0	167.9	84.0	84.0
	September	0	158.3	79.2	79.2
	October	71.8	116.3	58.2	129.9
	November	16.3	22.0	11.0	27.2
	Total	88.0	563.9	282.0	370.0

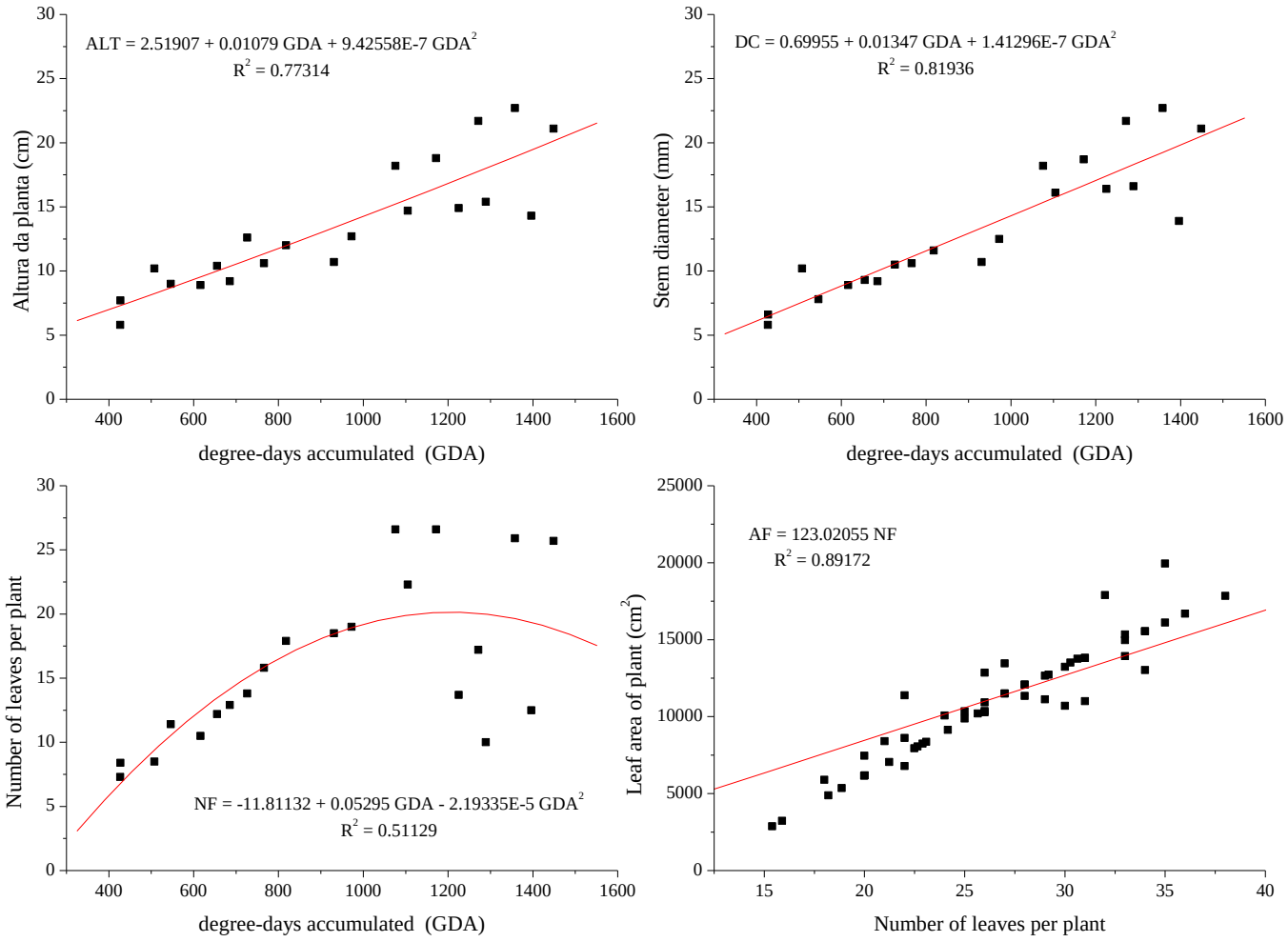

Figure 2. Estimates of the morphometric variables of cauliflower plants cv. Verona based on the accumulated thermal sum, by taking into consideration the two cultivation cycles (2014 and 2015) in Sinop-MT, Brazil.

Table 4. Morphometric variables of cauliflower plants cv. Verona under different irrigation blades and development phases, between August 21st and November 26th, 2014, in Sinop-MT, Brazil.

DAT	GDA	Plant height (cm)				Stem diameter (mm)				Number of leaves			
		L60*	L80*	L100*	L120*	L60	L80	L100	L120	L60	L80	L100	L120
32	426.9	7.1 ^{a}	6.6 ^{a}	3.8 ^{a}	5.6 ^{a}	3.9 ^{a}	3.9 ^{a}	3.7 ^{a}	3.2 ^{a}	8.1 ^{a}	7.3 ^{a}	6.7 ^{a}	6.9 ^{a}
39	507.6	9.6 ^{ab}	8.0 ^{b}	11.8 ^{a}	11.4 ^{a}	4.2 ^{a}	4.4 ^{a}	4.1 ^{a}	4.0 ^{a}	8.4 ^{a}	8.3 ^{a}	8.5 ^{a}	8.6 ^{a}
47	616.2	10.0 ^{a}	8.5 ^{a}	9.0 ^{a}	8.3 ^{a}	7.9 ^{a}	7.4 ^{a}	7.5 ^{a}	6.6 ^{a}	10.7 ^{a}	10.6 ^{a}	11.0 ^{a}	9.7 ^{a}
54	685.2	9.7 ^{a}	8.8 ^{a}	9.5 ^{a}	8.9 ^{a}	10.0 ^{a}	8.8 ^{a}	9.1 ^{a}	8.4 ^{a}	13.3 ^{a}	12.8 ^{a}	13.2 ^{a}	12.4 ^{a}
61	766.1	11.7 ^{a}	9.6 ^{a}	11.2 ^{a}	9.9 ^{a}	11.9 ^{a}	11.7 ^{a}	12.1 ^{a}	10.8 ^{a}	17.2 ^{a}	15.4 ^{a}	16.2 ^{a}	14.2 ^{a}
77	931.0	12.5 ^{a}	9.4 ^{a}	11.6 ^{a}	9.6 ^{a}	14.7 ^{a}	12.7 ^{a}	14.3 ^{a}	12.1 ^{a}	20.4 ^{a}	18.6 ^{a}	17.5 ^{a}	17.6 ^{a}
89	1076.0	19.3 ^{a}	17.6 ^{a}	18.9 ^{a}	17.2 ^{a}	23.6 ^{a}	20.4 ^{a}	21.6 ^{a}	19.2 ^{a}	28.5 ^{a}	26.4 ^{a}	27.0 ^{a}	24.6 ^{a}
96	1171.8	19.7 ^{a}	19.9 ^{a}	19.1 ^{a}	16.4 ^{a}	26.9 ^{a}	23.1 ^{a}	25.1 ^{a}	22.3 ^{a}	25.6 ^{a}	26.7 ^{a}	28.4 ^{a}	25.5 ^{a}
103	1271.6	24.2 ^{a}	20.8 ^{ab}	23.1 ^{a}	18.6 ^{b}	28.9 ^{a}	26.8 ^{a}	27.9 ^{a}	23.7 ^{a}	29.2 ^{a}	26.7 ^{ab}	28.6 ^{ab}	24.2 ^{b}
110	1357.9	24.3 ^{a}	22.1 ^{a}	22.6 ^{a}	21.7 ^{a}	36.3 ^{a}	31.7 ^{a}	34.2 ^{a}	30.8 ^{a}	26.8 ^{a}	26.2 ^{a}	24.8 ^{a}	25.8 ^{a}
117	1449.0	23.5 ^{a}	20.6 ^{ab}	18.4 ^{b}	21.8 ^{ab}	29.6 ^{a}	27.7 ^{a}	29.6 ^{a}	25.1 ^{a}	26.3 ^{ab}	27.0 ^{a}	22.4 ^{b}	26.9 ^{a}

*L60, L80, L100 and L120 correspond to the evapotranspiration reposition rate (irrigated blade) of the culture (ETc). Means followed by the same letter on the line (analysis of irrigation blade influence at the same evaluation day) did not differ from each other in the Tukey test at 5% probability.

morphometric variable or treatment, when values were compared to that of 2014; however, such result did not indicate yield decrease. Thus, such performance can be explained by the significant increase in cloudiness and i rainfall rates from October, 2014 and after. A significant change in the number of leaves between 77 and 89 DAT was observed. It is worth highlighting that solar radiation incidence on the surface of Earth is the main energy source for vegetal development, mainly for leaves, which need PAR (photosynthetic active radiation – one of the global radiation components) to make photosynthesis as the primary metabolism process in plants.

The irrigation blades led to significant differences in plant height, stem diameter and number of leaves at 5% probability (Table 5) in the 2015 crop, from the 73, 87 and 100 DAT and after, respectively. There was increase in the number of leaves, up to the 87 DAT, and further reduction (senescence) was noticed due to the beginning of the reproductive phase. Such performance is inherent to the cycle of this vegetable, which presents fast leaf-surface growth in order to allow greater production of photo-assimilates in the reproductive phases, and subsequent decrease after this phase.

The stem can be considered as an essential partition for plants, since it holds the conducting beams (xylem and phloem) responsible for taking water from the root system to the shoot, as well as for distributing the photo-assimilates to the entire body of the plant, besides being the support structure to the shoot. Thus, there was initial growth up to the 109 DAT and subsequent reduction due to wilting at the end of the plant cycle. The increase in stem diameter, and in plant height, highlight the lack of high plant densities; therefore, the adopted spacing, and the association of the local micro-meteorological conditions with the employed culture management, did not favor the outspread of the main physiological diseases and disorders such as hollow stalk and tenderness.

Oliveira (2015) analyzed the irrigation blades and the N fertilization for the cauliflower cultivar Barcelone and found stronger irrigation blade effect than fertilization effect. He recorded higher culture development indices by using irrigation equivalent to 132.4% Tec in Minas Gerais State. Similar results were recorded by Seidel et al. (2017), who observed that the maintenance of the field capacity until the end of the final formation of cabbage head (*Brassica oleracea* L. var. Capitata) was favorable to good culture yield; however, stress caused by water deficit reduced the plant height and the size of the heads. Results of both cultivation cycles showed that the initial effects of possible water stress could be compensated by plant adaptive responses to the cultivation environment. Plants can have different responses to different water deficits, since there are the tolerant ones, which overcome stress by changing their morphological and biochemical features, and the susceptible ones, which develop symptoms resulting in lower yield (Chakraborty et al., 2015).

Overall, when plants are exposed to water deficit conditions, there are physiological responses that can indirectly result in water conservation in the soil due to decrease in the transpiration surface through senescence, reduced expansion or leaf elongation, which in their turn, depend on water turgescence and availability to the plants. Bergamaschi and Bergonci (2017) state that low water availability in the soil can cause leaf area reduction and lead to decreased photosynthetic capacity and to lower growth and yield rates. The regressions were adjusted based on thermal sums as independent variables in order to assess the effect of cultivation cycle duration (“time” as quantitative variable) (Figure 2). In this case, regardless of the assessed morphological

Table 5. Morphometric variables of cauliflower plants cv. Verona under different irrigation blades and development phases between July 11th and November. 07th, 2015, Sinop-MT, Brazil.

DAT	GDA	L40*	L60*	L80*	L100*	L120*
			Plant height (cm)			
32	427.2	7.7 a	8.1 a	7.5 a	7.5 a	7.7 a
41	546.0	9.5 a	9.3 a	8.6 a	9.1 a	9.6 a
48	655.2	10.7 a	11.2 a	9.8 a	11.2 a	9.1 a
53	726.4	12.4 a	11.6 a	12.0 a	11.0 a	11.9 a
62	818.1	12.2 a	11.5 a	11.5 a	12.4 a	12.5 a
73	972.5	12.2 a	12.9 a	11.8 a	13.5 a	13.1 a
87	1105.2	13.7 a	15.0 a	13.7 a	16.1 a	14.8 a
100	1225.1	13.1 a	15.9 a	13.4 a	16.4 a	15.5 a
109	1288.9	12.0 b	16.7 a	14.3 ab	17.6 a	16.6 a
119	1396.4	11.5 b	15.6 a	10.6 b	17.0 a	16.8 a
			Stem diameter (mm)			
32	427.2	6.5 a	6.67 a a	6.44 a	6.89 a	6.65 a
41	546.0	8.3 a	7.78 a	7.25 a	7.81 a	8.05 a
48	655.2	9.6 a	9.57 a	8.61 a	9.56 a	8.99 a
53	726.4	10.7 a	10.97 a	11.18 a	9.21 a	10.65 a
62	818.1	12.2 a	11.6 a	10.85 a	12.26 a	11.33 a
73	972.5	12.5 a	11.94 a	12.04 a	13.58 a	12.21 a
87	1105.2	15.0 a	17.6 a	14.93 a	17.96 a	15.11 a
100	1225.1	14.2 b	18.42 a	16.37 ab	17.17 ab	15.86 ab
109	1288.9	14.2 b	19.2 a	18.44 a	18.75 a	15.33 b
119	1396.4	11.7 b	16.21 a	10.65 c	16.58 a	14.66 ab
			Number of leaves			
32	427.2	8.1 a	8.4 a	8.0 a	8.9 a	8.9 a
41	546.0	11.3 a	11.6 a	10.3 a	12.2 a	11.5 a
48	655.2	12.0 a	12.4 a	11.3 a	12.6 a	12.9 a
53	726.4	12.9 a	15.6 a	14.7 a	12.5 a	13.6 a
62	818.1	17.8 a	18.6 a	17.4 a	18.0 a	17.9 a
73	972.5	17.0 b	21.5 a	18.0 ab	20.5 ab	18.2 ab
87	1105.2	22.2 ab	24.0 a	19.8 b	22.3 ab	23.4 ab
100	1225.1	10.6 b	15.9 a	13.3 ab	15.0 a	13.6 ab
109	1288.9	6.4 b	12.0 a	9.4 ab	11.7 a	10.5 a
119	1396.4	6.5 c	13.0 a	11.0 ab	10.5 b	10.5 b

*L40, L60, L80, L100 and L120 correspond to the evapotranspiration reposition rate (irrigated blade) of the culture (ETc). Means followed by the same letter on the line (influence analysis of irrigation blades in a single development phase) did not differ from each other in the Tukey test at 5% probability.

variables, the coefficients adjusted at the linear (Figure 2) and quadratic (Figure 2) functions were significant at 1.0% probability; correlation coefficients (r) were higher than 71.5%.

The cauliflower cv. Verona presented inflorescence variation from 41 to 53 DAT under Sinop-MT weather conditions, regardless of the applied irrigation management. Inflorescences presented diametric ratios (ratio between equatorial and longitudinal diameter) varying from 1.01 and 1.0; thus, indicating flat shapes (Table 6). There were significant differences in the equatorial and longitudinal diameters of inflorescences under the different irrigation blades, and it allowed assuming that there was harvest point standardization. Silva et al. (2014) found inflorescence diameter from 19.86±0.82 to 21.49±0.30 cm in cauliflowers cultivated under the different irrigation systems. Similar results were also recorded by Kudela et al., (2011), who assessed the quality of cauliflower under 2 different irrigation blades and found ID from 19.35 to 20.05 cm. Overall, bigger inflorescences are more valorized in the market, for they comply with the higher classes in the

Table 6. Productive components of cauliflower plants cv. Verona under different irrigation blades and development phases, by taking into consideration the two cultivation cycles (2014 and 2015) in Sinop-MT, Brazil.

Year	Irrigation blades	DINF (days)	DEq (cm)	DLo (cm)	IFM (g plant^{-1})	AF (cm²)
2015	L40	46.3 [a]	20.6 [a]	19.63 [a]	488.75 [b]	11041.4 [b]
	L60	46.9 [a]	21.4 [a]	20.25 [a]	563.98 [b]	12800.2 [ab]
	L80	43.6 [a]	24.0 [a]	21.94 [a]	730.66 [a]	11727.2 [b]
	L100	49.9 [a]	24.8 [a]	22.75 [a]	871.51 [a]	14678.0 [a]
	L120	41.3 [a]	22.7 [a]	20.5 [a]	751.4 [a]	14733.7 [a]
	DMS	15.2	5.0	5.18	157.4	3214.7
	CV(%)	23.1	16.3	20.74	16.1	27.91
2014	L60	50.0 [a]	20.5 [a]	20.20 [a]	435.7 [a]	8368.1 [a]
	L80	46.0 [a]	20.8 [a]	20.29 [a]	470.1 [a]	8763.3 [a]
	L100	53.0 [a]	21.1 [a]	20.42 [a]	514.3 [a]	7746.5 [a]
	L120	44.0 [a]	20.8 [a]	20.31 [a]	477.03 [a]	7048.8 [a]
	DMS	12.4	2.4	0.82	286.9	2638.5
	CV(%)	20.4	5.4	1.91	28.8	25.57

normal Brazilian classifications for trading standards (Hortibrasil, 2018). However, there is great variability in this feature in the same cultivar (Monteiro et al., 2010; Torres et al., 2015; Ribeiro et al., 2017).

The inflorescence fresh mass (IFM) presented significant difference between irrigation blades in the 2015 cultivation cycle: variations higher than 380 g at 40% irrigated blade increase. This behavior highlights that although there were no diametric differences, the irrigated blade influenced the density of immature inflorescences. Regardless of cultivation cycle and irrigated blade, there were inflorescences presenting white-milky color and formed cover (2 leaves tight to the edges). These features are bond to the assessed cultivar. Ribeiro et al. (2017) found that in tropical conditions Verona CMS is more tolerant to high temperature than cvs. Sarah and Sharon, exhibiting more marketable products on postharvest by reducing hollow stem and physiological disorder. It is also possible to highlight the record of less than 5% severe defects, such as the presence of leaves in the head (leaf emergence in the internal part of the inflorescence), hairiness (opened flowers in the head, similar to hair), purple spots (pinkish spots resembling wine on the inflorescence) and hollow stalk. Monteiro et al. (2010) assessed the water performance of summer cauliflowers and found IFM from 0.73 to 1.11 kg.

With regard to inflorescence commercial yield, there was quadratic performance with the irrigated blades in the 2 cultivation cycles. In 2014, the generated functions were Prod = 2326.58 + 35615.3 LI – 17917.5 LI^2; and in 2015 they were Prod = 2735.28 + 64257.7 LI – 29750.7 LI^2. The determination coefficients (R^2) were 0.86 and 0.88 (in this case, LI is the irrigated blade in decimals L40, L60, L80, L100 and L120, which corresponded to 0.4, 0.6, 0.8, 1.0 and 1.2, respectively). The maximum points for water reposition at 99.34 and 107.8% of ETc were found by applying the derivative of these polynomials, that is, irrigation management with daily reposition close to the 100% ETc recommended by the Class A tank method. Such values allow better yield results for cauliflower cv. Verona (Table 7). It is also possible to mention that the increased rainfall after 60 DAT in 2014 in comparison to 2015 enabled significant reduction in water use efficiency (EUA), even when only irrigation was taken into account. Air temperature variations and global radiation (Figure 1) indicated that it is possible to find positive correlation between commercial yield and the micro-meteorological elements in this genetic material, that is more stable thermal amplitudes through the cycle tend to favor better inflorescence development and, consequently, more fresh weight (Zanuzo et al., 2013). The recorded yields are similar to that recorded by Monteiro et al. (2010): mean yield between 14.56 and 23.76 t ha^{-1} in different genetic materials of the summer cycle. Zanuzo et al., (2013) found mean yield of 18.9 and 18.7 t ha $^{-1}$ for hybrids Verona 184 and Verona 284 in the Sinop region. Pereira et al. (2016) noted that desert hybrid (summer cultivar, with a cycle varying between 80 and 90 days) is promising for cultivation in the soil and climatic conditions of the region Amazon; its productivity is 17.1 t ha^{-1}, it has inflorescence fresh mass of 0.85 kg plant^{-1}, inflorescence diameter of 18 cm, and 38 kPa tension of soil water content.

Conclusion

It is recommended to adopt daily water reposition

Table 7. Mean yield and water use efficiency (kg m^{-3}) of cauliflower cv. Verona CMS, subjected to different irrigation blades in Sinop-MT, Brazil.

Year	Irrigation blades	Productivity (kg ha^{-1})	Irrigation depth - I (mm)	I + Pef	EUA^{1*}	EUA^{2*}
2015	L40	19,550.0	112.8	200.8	17.33	9.74
	L60	22,559.2	169.2	257.2	13.33	8.77
	L80	29,226.4	225.6	313.6	12.95	9.32
	L100	34,860.4	282.0	370.0	12.36	9.42
	L120	30,056.0	338.4	426.4	8.88	7.05
2014	L60	17,428.0	166.7	591.5	10.45	2.95
	L80	18,804.0	222.2	647.0	8.46	2.91
	L100	20,572.0	277.8	702.6	7.41	2.93
	L120	19,081.2	333.3	758.1	5.72	2.52

EUA^1 and EUA^2 take into consideration the irrigation blade and the irrigation blade + effective rainfall, respectively.

between 80 and 100% of the evapotranspiration of the culture in winter-fall cauliflower crops in Middle-Northern Mato Grosso State. The irrigation blade variations did not influence the duration and thermal demands of the differentiation period of cauliflower cv. Verona CMS inflorescences. Increased irrigated blades reduced water use efficiency, regardless of cultivation cycle: it produced from 7.4 to 12.4 kg of inflorescence fresh mass per m^3 of irrigated water.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Allen RG, Pereira LS, Raes D, Smith M (1998). Crop evapotranspiration: guidelines for computing crop water requirements. Rome, FAO (Irrigation and Drainage Paper, 56). 300 p.

Bergamaschi H, Bergonci JI (2017). As plantas e o clima – princípios e aplicações. 1 ed. Agrolivros, 352p.

Cardoso AII, Silva N (2009). Influência do cultivar e do tamanho das sementes na produção de couve-flor. Revista Ceres 56(6):777-782.

Chakraborty K, Singh, A, Kalariya KA, Goswami N, Zala PV. Physiological responses of peanut (*Arachis Hypogaea* L.) cultivars to water déficit stress: status of oxidative stress and antioxidante enzyme activities. Chakraborty 74(1):123-142.

Claudio MTR (2013). Doses de fósforo no acúmulo de nutrientes, na produção e na qualidade de sementes de couve-flor. Dissertação, UNESP, Botucatu 66p.

Domínguez A, Martínez-Navaro A, López-mata E, Tarjuelo JM, Martínez-Romero A (2017). Real farm management depending on the available volume of irrigation water (part I): Financial analysis. Agricultural Water Management 192:71-84.

EMBRAPA (2013). Empresa Brasileira de Pesquisa Agropecuária. Centro Nacional de Pesquisa de solos. Sistema brasileiro de classificação de solos. 3.ed. Rio de Janeiro: Embrapa, 353p.

Filgueira FAR (2005). Novo Manual de Olericultura. Viçosa: UFV, pp. 269-288.

Garcia Filho E, Nakatani JK, Pinto MJA, Neves MF, Caserta PG, Kalaki RB, Gerbasi T (2017). Mapeamento e qualificação da cadeia produtiva das hortaliças do Brasil. Confederação da Agricultura do Brasil. Brasília: CNA 79 p.

Hortibrasil (2018). Instituto Brasileiro de Qualidade em Horticultura. Normas de Identidade, Padronização e Classificação da Couve-flor. 2018. Disponível em: < http://www.hortibrasil.org.br/classificacao/couveflor/couveflor.html>. Acessado em 20 de março de 2018.

IBGE (2010). Instituto Brasileiro de Geografia e Estatística, Censo Demográfico. Acesso: 11 dez. 2017. Disponivel em: https://cidades.ibge.gov.br/painel/populacao.php?codmun=510790

Kochler M, Kage H, Stützel H (2007). Modelling the effects of soil water limitations on transpiration and stomatal regulation of cauliflower. European Journal of Agronomy 26:375-383.

Koksal S, Tasan M, Artik C, Gowda P (2017). Avaliação da eficiência financeira da irrigação por gotejamento de pimenta vermelha com vase na evapotranspiração calculada usando uma abordagem interativa de orçamento de água no solo. Scientia Horticulturae 226:398-405.

Kudela M, Hnilička F, Svozilová L, Martinková J (2011). Cauliflower qualities in two irrigation levels with the using of hydrophilic agent. HortScience 38(2):81-85.

Léllis BC, Carvalho DF, Martínez-Romero A, Tarjuelo JM, Domínguez A (2017). Effective management of irrigation water for carrot under constant and optimized regualted déficit irrigation in Brazil. Water Management 192:294-305.

May A, Tivelli SW, Vargas PF, Samra AG, Sacconi LV, Pinheiro MQ (2007). A Cultura da Courve-Flor, Campinas: Instituto Agronômico, (Série Tecnologia APTA, Boletim Técnico IAC, n. 200), 36p.

Monteiro BCBA, Charlo HCO, Braz LT (2010). Desempenho de híbridos de couve-flor de verão em Jaboticabal. Horticultura Brasileira 28:115-119.

Morais Junior OP, Cardoso AF, Leão EF, Peixoto N (2012). Performance of cultivars of cauliflower summer in Ipameri. Ciência Rural 42(11):1923-1928.

Nowbuth RD, Pearson S (1998). The effect of temperature and shade on curd initiation in temperate and tropical cauliflower. Acta Horticulture 459:79-88.

Oliveira RM (2015). Produção das culturas do Brócolis e da Couve-Flor

com diferentes lâminas de irrigação e doses de nitrogênio. Dissertação, UFV, Viçosa 74p.
Pedrosa TD, Ozima HT, Schneider RM, Souza AP, Andrade EA, Mattos LV (2017). Phosphorus, copper and zinc leached in lysimeters with red-yellow latosol subjected to diferente rates of reused swine water na irrigation water. African Journal of Agricultural Research 12(39):2902-2909.
Pereira MEM, Lima Junior JA, Souza RORM, Gusmão SAL, Lima VM (2016). Irrigation management influence and fertilizer doses with boron on productive performance of cauliflower. Engenharia Agrícola 36(5):811-821.
Puiatti M, Finger FL (2009). Fatores climáticos. In: Fontes PCR (Org.). Olericultura: teoria e prática. 1ed.Visconde do Rio Branco: Suprema Gráfica e Editora, v. único, pp. 17-30.
Ribeiro LMP, Zanuzo MR, Vieira CV, Seabra Junior S, Fernandes Júnior F (2017). Cauliflower quality and yield under tropical conditions are influenced by boron fertilization. African Journal of Agricultural Research 12(12):1045-1053.
Sahin U, Kuslu Y, Tunc T, Kiziloglu FM (2009). Determining crop and pan coefficicients for cauliflower and red cabbage crops under cool season semiarid climatic conditions. Agricultural Sciences in China 8(2):167-171.
Santos RB, Souza AP, Silva AC, Almeida FT, Arantes KR, Siqueira JL (2013). Planejamento da pulverização de fungicidas em função das variáveis meteorológicas na região de Sinop-MT. Global Science and Technology 6(1):72-88.
Sarkar S, Biswas M, Goswami SB, Bandyopadhyay PK (2010). Yield and water use efficiency of cauliflower under varying irrigation frequencies and water application methods in Lower Gangetic Plain of India. Agricultural Water Management 97:1655-1662.
Sarkar S, Nanda MK, Biswas M, Mukherjee A, Kundu M (2009). Different indices to characterize water use pattern of irrigated cauliflower (*Brassica oleracea* L. var. botrytis) in a hot sub-humid climate of India. Agricultural Water Management 96:1475-1482.
Seidel SJ, Werisch S, Schutze N, Laber H (2017). Impact of irrigation on plant growth end development of White cabbage. Agricultural Water Management 187:99-112.
Silva FL, Almeida ACS, Geisenhoff LO, Oliveira FC, Pusch M, França SLS (2014). Produtividade da couve-flor sob diferentes sistemas de irrigação. In: Inovagri International Meeting, II. DOI: http://dx.doi.org/10.12702/ii.inovagri.2014-a213
Sousa VF, Marouelli WA, Coelho EF, Pinto JM, Coelho Filho MA (2011). Irrigação e fertirrigação em fruteiras e hortaliças. Brasília, DF: Embrapa Informação Tecnológica 771 p.
Souza AP, Almeida FT, Arantes KR, Martim CC, Silva JO (2015). Coefficients of Class A tank to estimate the daily reference evapotranspiration in the Cerrado-Amazonian transition region. Scientia Plena 11(05):01-11.
Souza AP, Mota LL, Zamadei T, Martim CC, Almeida FT, Paulino J (2013). Climatic classification and climatological water balance in the state of Mato Grosso. Nativa 1(1):34-43.
Souza AP, Pereira JBA, Silva LDB, Guerra JGM, Carvalho DF (2011a). Evapotranspiração, coeficientes de cultivo e eficiência do uso da agua da cultura do pimentão em diferentes sistemas de cultivo. Acta Scientiarum Agronomy 33(1):15-22.
Souza AP, Ramos CMC, Lima AD, Florentino HO, Escobedo JF (2011b). Comparison of methodologies for degrre-day estimation using numerical methods. Acta Scientiarum Agronomy 33(3): 91-400.
Souza AP, Zamadei T, Monteiro EB, Casavecchia BH (2016). Transmissividade atmosférica da radição global na região amazônica de Mato Grosso. Revista Brasileira de Meteorologia 31(4):639-648.
Tomassoni F, Santos RF, Rocha AA, Galdino TS, Nadaleti WC, Rossi E, Carpinski M (2013). Sensibilidade da couve-flor ao excesso de água no solo. Acta Iguazu 2(4):1-6.
Torres JLR, Araújo AS, Barreto AC, Silva Neto OF, Silva VR, Vieira DMS (2015). Desenvolvimento e produtividade de couve-flor e repolho influenciados por tipos de cobertura do solo. Horticultura Brasileira 33(4):510-514.
Yavuz D, Seymen M, Yavuz N, Türkmen Ö (2015). Effects of irrigation interval and quantity on the yield and quality of confectionary pumpkin grown under field conditions. Agricultural Water Management 159:290-298.
Zanuzo MR, Ribeiro LM, Lange A, Machado RAF, Massaroto JA (2013). Desempenho agronômico de genótipos de couve-flor nas condições edafoclimáticas de Sinop. Horticultura Brasileira 31:332-337.

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Comparative analysis of antioxidant activities of different varieties of mangos with some selected fruits

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Natural antioxidants and secondary metabolites found in vegetables and fruits play significant role in human health. The juices obtained from fruits and vegetables are more convenient to consume than fresh vegetables and fruits. In the current study, the total phenolic, vitamin C, total carotene and B-carotene content and antioxidant activity of fruits and vegetables juices were determined by 1,1-diphenyl-2picryl hydrazyl (DPPH) scavenging, 2,2–azinobis3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and reducing power (RP) assays. Various fruits juices such as Chinese mango juice (Ao mango, Ao flim package, Xiangya mango) and among (Apple, Orange, Tomato, Carrot) juices were compared. Among the fruits, the highest amount of total phenolic (328.18 mg/100 g) and vitamin C (46.8 mg/100 g) content was found in orange juice among all the other fresh juices. Among different mango juices, the highest amount of total phenolic (145.52 mg/100 g) and vitamin C (16.4 mg/100 g) content was found in Xiangya mango as compared to Ao mango and Ao (flim package) mango. While Ao (flim package) mango contained the highest amount of total carotene (2771 µg/100 g) and B-carotene (1.955 mg/100 g), as compared Ao mango and Xiangya mango. The highest amount of total carotene (6062 µg/100 g) and B-carotene 5.398 mg/100 g was found in carrots as compared to the fruit and vegetable juices. However, the lowest content of total phenolic 15.1 mg/100 g and vitamin C (1.87 mg/100 g) was found in carrots as compared to other fruits and vegetable juices. The highest antioxidant activity was recorded in Xiangya mangoes and oranges juice through DPPH, ABTS, and RP scavenging assays as compared to other Juices. However, the lowest antioxidant activity was recorded in carrot juice. The present study demonstrates the potential value of fruit juice as their placement of fresh fruit.

Key words: Chinese mango, phenolic compounds, B-carotene, antioxidant activity.

INTRODUCTION

China is one of the leading mangoes exporting countries in the world with potential local as well as internal markets. The climatic environment permits the cultivation of mangoes throughout the year by the use of stimulation

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techniques particular flower. It is believed that factors like cultivar, agronomic conditions, post-harvest manipulation and stage of ripeness significantly affect the antioxidant properties of mangoes (Lemmens et al., 2013). Mango (*Mangifera indica* L.) is regarded as the king of all fruits, possessing rich dietary source, antioxidants, such as vitamin C, vitamin E, as well as carotenoids and phenolic compounds, which have shown various health effects on the human body (Barbosa et al., 2017; Liu et al., 2014). Recent study showed that the mango juice has possibility as a functional food that is capable of reducing metabolic risk for obesity associated with adiposity and inflammation (Natal et al., 2017). Mango is considered as one of the most popular and economically important fruits due to its admirable sensorial characteristics (sweet taste, bright color, and delicious flavor), as well as nutritional composition (carbohydrate such as abundant glucose, fructose and sucrose, vitamins, minerals, fiber, and photochemical (Barbosa et al., 2017). Mangoes provide excellent quantity of antioxidant activity and bioactive compounds, playing a beneficial role in human health. These bioactive compounds are helpful in preventing different pathological conditions like cardiovascular disease and decreasing the risk of various types of cancers and atherosclerosis (Nemec et al., 2017; Ulla et al., 2017). The antioxidant potential of different vegetable and fruits is associated with their carotenoids composition, and/or total phenolic content (Liu et al., 2014). Although, total phenolic compounds and antioxidant activity of mangoes and other fruits have been extensively studied; however, there is still lack of information regarding composition and changes in these phenolic compounds and their antioxidant potential during the ripening period (Lee and Hwang, 2017). The literature available on this reveals that these phenolic compounds have varied antioxidant potential, depending upon the number of hydroxyl groups and their distribution in the structure (Heo et al., 2007). It is well known that reactive oxygen species (ROS), like superoxide anion radical, hydroxyl radical and hydrogen peroxide are formed in the human body through enzymatic systems during oxygen utilization (Ahmed et al., 2017). A small quantity of these ROS is thought to be favorable for the body performing different vital roles such as transfer of neuro signal from one location to another (Afifa et al., 2014) and also in growth regulator (Carlis et al., 2014). Nevertheless, huge quantity of ROS are known to be involved in causing different pathologic conditions in the human bodies, such as cancer, cardio vascular diseases (CVD), aging and neurodegenerative diseases (Lauricella et al., 2016). Therefore, the firm capacity of exogenous antioxidants is continually required to sustain a sufficient amount of antioxidants in order to stabilize the ROS. Similarly, mangoes have particularly rich source of polyphenols, which are a various group of natural micronutrients present in plants having specific health benefits, that is, mangiferin is antioxidant compounds which may encourage metabolism and scrap diabetes and quercetin, which can decrease inflammation and hypertension as well as reduce cancer growth (Nemec et al., 2017). However, because of different mechanisms of reaction characteristics occupied, one analysis is capable of perfecting all the analysis of antioxidants in a miscellaneous or complex system. Therefore, in order to evaluate a full profile of the potential antioxidant, various assays capacity of antioxidant may be compulsory. In this context, different technique has been applied to determine antioxidants in biological fluids, food materials, nutraceuticals, and dietary supplements (Emoke et al., 2010). Among these methods, reducing power, 2, 2-azino- di-(3-ethylbenzothialozine-sulphonic acid) (ABTS assay), 2, 2-diphenyl-1-picrylhydrazyl (DPPH assay), and hydroxyl radical scavenger activity are the commonly used antioxidant capacity assays. These methods are different from everyone both in conditions of their evaluation roles as well as experimental situation. The main aim of this study is to evaluate the antioxidant properties of different varieties of mangos and compare them with some selected fruits.

MATERIALS AND METHODS

Chemicals and reagents

DPPH, ABTS, potassium peroxy-di-salfate ($K_2S_2O_8$), ascorbic acid, potassium ferricyanide, trichloroacetic acid, ferric chloride, phosphate buffer solution (PBS), gallic acid, Folin-Ciocalteu's, ascorbic acid, methanol, acetone, methylene chloride and ethanol were purchased from Sino Pharma-Chemical Reagents Co, Ltd. (Shanghai, China). All the chemicals used were of analytical grade and were used without further purification.

Preparation of fruit juices

Mangoes were purchased from Hainan province, and the other fruits were purchased from the local supermarket of Huazhong Agricultural University, Wuhan, Hubei province, China. Mangoes, other selected fruits (apple, orange, tomato) and vegetable (carrot) were washed with tap water and dried, followed by cutting into small pieces separating pulp from peel. Afterward, pulp and fruit juice were extracted by using juice extractor (AUX-PB953).

Determination of Brix, pH, and acidity

Brix was measured using WYT-80 hand refractometer (Quanzhou Wander Experimental Instrument Co., Ltd., Quanzhou, China). The pH was measured using a digital pH meter (Delta 320 pH meter, Metller Toledo Instruments Co., Ltd., Shanghai, China), while titratable acidity was measured according to the method suggested by the "Association of Official Analytical Chemists" (AOAC, 2000). The samples were titrated with N/10 NaOH solution using titration kit, where phenolphthalein (3-5 drops) was used as an indicator. The volume of alkali used was noted and calculation was made using the following formula:

Titratable acidity (%) = Quantity of N/10 NaOH used × 0.009 / Volume of sample taken

Determination of total phenols content

To determine the total phenols content, 100 µL sample was mixed with 0.4 mL distilled water (ddH_2O) and 0.5 mL diluted Folin-Ciocalteu reagent. The samples were incubated for 5 min and then 1 mL 7.5% sodium carbonate (w/v) was added. The absorbance was measured at 765 nm using spectrophotometer. The results were obtained as mg of gallic acid equivalents/100 g of fresh sample (Musa et al., 2011).

Determination of vitamin C

Vitamin C content for the fruit juices were determined using protocol of Dashman et al. (1996) with some modifications. About 20 µL of sample was pipetted into a 100-mL volumetric flask. Then 2 mL (10%) tetra-chloro-acetic acid solution was added. The solution was diluted up to 100 mL with distilled water. The sample was poured into a conical flask, swirled gently for 1 min and left to stand for 1 min, and filtered through a Whatman filter (No. 542). One milliliter of the sample or standard solution (3 mg ascorbic acid in 1 mL distilled water) was pipetted into a test tube, followed by the addition of 3 mL distilled water and 0.4 mL of Folin-Ciocalteu reagent. Afterward the samples were incubated at room temperature for 10 min. The absorbance was measured at 760 nm using the Unico™ UV-2100 spectrophotometer. The results were expressed as mg/100 g fresh weight (FW).

Determination of total carotenoids

Total carotenoids were determined using the method of Ranganna et al. (1999). Approximately, 5 g of sample was added to 20 ml of acetone and maintained in the dark for 10 to 15 min. Afterwards, the contents were filtered through a sintered funnel under suction. About 20 ml of acetone was added twice and then 20 ml of hexane was added to extract the pigment completely. The mixed solutions were transferred to a separating funnel. After 5 min, the upper aqueous layer was completely discarded. However, the lower hexane layer was transferred to 250-ml volumetric flask. The volume of flask was filled up to the mark with hexane. Slight amount of anhydrous sodium sulfate was added and the absorbance was measured at 450 nm against hexane as a blank. The carotenoid content of each sample was estimated according to the following equation:

Absorbance × 250 × 1000 × 100 / 250 × Weight of the sample.

The carotene was expressed as µg/100 mL.

Determination of β-carotene

HPLC method was used for determination of β-carotene according to the method of Hymavathi et al. (2005). Sample (15 g) was added to 30 mL acetone. The samples were sonicated for 30 min followed by centrifugation at 9000 rpm at 4°C for 15 min. The procedure was repeated twice to ensure the maximum extraction. The extracts were collected and made up to final volume of 100 mL by adding distilled water. After that, 50 mL of methanolic KOH (10%) was added to the extracts for saponification at 45°C water-bath for 1 h. Then, the extracts were transferred to 100 mL petroleum ether, and the organic layer was dried by passing through an anhydrous sodium sulphate column and evaporated to dryness. The dried residue was dissolved in hexane and filtered through a 0.45 µm membrane filter. The filtrate was analyzed with the RF-10AXL HPLC system (Shimadzu Co., Ltd., Japan) carried out at 445 nm at 30°C. The analytical column was a Sunfire™ C18 (4.6×250 mm i.d., 5 µm particle size) from waters. The mobile phase was acetonitrile: methanol: methylene chloride (6:2:2, v/v/v), with isocratic flow at a rate of 1.0 mL/min. The concentration was calculated using β-carotene as external standard and expressed as microgram β-carotene per 100 g of FW.

DPPH radical scavenging activity assay

The DPPH free radical scavenging activity was observed according to the method described by Mendes et al. (2011). Briefly, 7 mg of DPPH was dissolved in 100 mL ethanol (95%). Different concentrations of each sample (mg/mL) were used from which 2 mL was mixed with 2 mL DPPH solution. Control was prepared by mixing 2 mL DPPH and 2 mL distilled water, whereas blank sample was prepared by mixing 2 mL sample with 2 mL ethanol. Then, samples were kept in the dark for 30 min. The absorption was measured at 517 nm. The samples were taken in triplicate and the results were expressed as means and standard deviation. The results were calculated for IC_{50} value.

ABTS radical scavenging activity

The ABTS free radical antioxidant activity was observed according to the method described by Re et al. (1999) with some modifications. Briefly, 6 mg ABTS and 2 mg potassium peroxi-disulfate ($K_2S_2O_8$) were dissolved in 1.47 and 2.86 mL distilled water, respectively. Then 1 mL of each ABTS and potassium peroxi-disulfate solutions were mixed together. The mixture was kept at dark for 12 h. Then 2 mL of mature was dissolved in 80 mL of 95% ethanol. From different concentrations of juice sample used (mg/mL), 150 µL of sample was mixed with 2.85 mL mixture solution. Control was prepared by mixing 150 µL distilled water and 2.85 mL mixture solution while blank mango by mixing 150 µL sample and 2.85 mL ethanol. The mixture was kept at 25°C for 5 min to ensure maximum reaction. The absorbance of each sample was measured at 734 nm. The samples were measured in triplicate and the results were expressed as mean ± standard deviation (SD). The results were calculated for IC_{50} value.

Reducing power

The reducing power was observed according to the method described by Lidia et al. (2011) with some modification. Different concentrations of sample extracts (1 mL) were mixed with 2.5 mL of 200 mM/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. The 2.5 mL of 10% trichloroacetic acid (w/v) was added and the mixture was centrifuged at 1000 rpm for 8 min in a refrigerated centrifuge (5805 AN 562248). Then 2.5 mL upper layer of each sample was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% of ferric chloride. The absorbance was measured at 700 nm by using Unico™ UV-2100 spectrophotometer.

Statistical analysis

The SPSS statistical package (IBM Statistics version 20) was used to analyze the results and determining significant difference. The results are presented as the mean value with the standard deviation (Mean ± SD). The significant difference was found using analysis of variance (ANOVA).

RESULTS

Brix, pH, and acidity

Table 1 results shows that the brix, pH, and acidity of

Table 1. pH, brix, titratable acidity, polyphenol, vitamin C, total carotene , B-carotene and lutenin content of mango and other fruits.

Name of fruits	pH	Brix	Titratable acidity (%)	Total polyphenol (mg GAE/100 g FW)	Vitamin C (mg/100g)	Total carotene (µg/100 g)	β-carotene mg/100 g	Lutein mg/100 g
Ao mango	4.86 ± 0.02^{b}	15.5 ±0.25^{a}	0.18 ± 0.01^{d}	102.7 ± 3.85^{d}	15.8 ±1.09^{c}	578 ± 14.4^{d}	0.467±0.11^{d}	0.111±0.03^{d}
Ao mango (film package)	4.78 ± 0.01^{b}	14.3 ± 0.12^{b}	0.18 ± 0.01^{d}	129.5 ± 3.75^{c}	15.8 ± 1.02^{c}	2771 ± 25.18^{b}	1.955±0.22^{b}	0.816±0.13^{a}
Xiang ya mango	4.72 ± 0.01^{b}	12.2 ±0.14^{c}	0.18 ± 0.01^{d}	145.52 ± 6.86^{b}	16.4 ± 0.97^{c}	1050 ± 29.03^{c}	0.672 ±0.15^{c}	0.378±0.08^{c}
Orange	3.54± 0.03^{e}	8.2 ± 0.08^{e}	0.82 ± 0.01^{a}	328.18 ± 7.91^{a}	46.8 ± 4.07^{a}	75 ± 9.01^{f}	0.075 ±0.02^{f}	-
Apple	3.98 ± 0.02^{d}	10.1 ± 0.12^{d}	0.54 ± 0.01^{b}	137.45± 11.9^{e}	18.6 ± 3.07^{c}	39 ± 6.40^{g}	0.034±0.03^{f}	0.005±0.01^{e}
Tomato	4.53± 0.01^{c}	2.4 ± 0.01^{g}	0.36 ± 0.01^{c}	75.66 ± 3.63^{f}	23.68 ± 3.6^{b}	247 ± 18.4^{e}	0.247±0.08^{e}	-
Carrot	6.8 ± 0.03^{a}	4.1 ± 0.02^{f}	0.09 ± 0.01^{e}	15.1 ± 0.19^{g}	1.87 ± 0.02^{d}	6062 ± 47.28^{a}	5.398 ±0.37^{a}	0.664±0.12^{b}

*Each value represents the means ± SEM. Triplicates in three independents experiments. Different letters within the column represents the significant difference (005).

"Ao" mango (15.5, 4.86 and 0.18), "AO" mango film (14.3, 4.78 and 0.18), Xiang-Ya" mango (12.2, 4.72 and 0.18), orange (8.2, 3.54 and 0.82), apple (10.1, 3.98 and 0.54), carrot (2.4, 4.53 and 0.36), and tomato (4.1, 6.8 and 0.09). The results indicated that "Ao" mango showed the highest brix value in all the experimental samples.

Spectrophotometric analysis of antioxidants compounds

Total polyphenol

Polyphenol compounds are very important fruit constituents due to their antioxidant activities, their chelation of redox-active metal ions, and inactivation of lipid free radical chains and prevention of hydroperoxide conversion into reactive oxyradicals (Cabral et al., 2009). Phenolic content can be used as an important indicator of antioxidant capacity and can be used as a preliminary screen for any product when intended to be used as a natural source of antioxidants in functional foods (Viuda et al., 2011). Total phenolic contents of fruits and vegetables juices are shown in Table 1. The highest quantity of phenolic contents (328.18 mg/100 g) was found in orange juice. However, carrots contain the lowest quantity of phenolic content (15.1 mg/100 g). Among the mango juices, the highest amount of polyphenol content (145.52 mg/100 g) was found in xiangya mango juice as compared to Ao mango and Ao (film package) mango juice.

Vitamin C

Vitamin C is a major antioxidant ingredient in must melon (Hoyle and Santos, 2010). Mango (*Mangifera indica* L.) being one of most consumed tropic fruit is rich in dietary antioxidants such as ascorbic acid, carotenoids and phenolic compounds. These compounds are involved in the protection of human against various diseases (Ribeiro et al., 2007).

Vitamin C content was found to be variable among the fruit and vegetable juices. Total vitamin C contents of various fruit and vegetable juices are shown in Table 1. The highest contents of vitamin C contents were found in 46.8 mg/100 g in fresh orange juice among all the test fruit and vegetable juices. However, carrot juice contains the lowest vitamin C content (1.87 mg/100 g) as compared to other fruit and vegetable juices. Vitamin C contents of mango juice of three varieties were also found to be highly variable. Among the mango varieties, the highest amount of vitamin C (16.4 mg/100 g) was found in xiangya mango when compared with the juices of Ao mango and Ao (film package) mango varieties.

Quantification of carotenoids by spectrophotometry and HPLC

Total carotene

Carotene specialized natural pigments and delivers colors such as yellow, orange and red (Perera and Yen, 2007). Apart from the spectacular colors in fruits and vegetables, these carotenoids in the diets are associated with the reduction of the diseases. Recent studies showed that diets high in carotenoids are important for health of human. Among the carotenoids the diets high in β-carotene and α-carotene are involved in the reduction of reduction of the incidence of type 2 diabetes (Sluijs et al., 2015).

Total carotene content in fruit and vegetable Juices are shown in Table 1. The highest of

carotene (6062 µg/100 g) was measured in carrot juices among all the test fruit and vegetable juices. Lowest total carotene content (75 µg/100 g) was measured in apple juices. Surprisingly among the different mango varieties juices, the highest amount of carotene contents (578 µg/100 g) was measured in the fresh Ao (flim package) mango as compared to Ao mango and xiangya mango juice. B-carotene content was found to be highly variable among the fruits and vegetable juices shown in Table 1. The highest amount of B-carotene (6.062 mg/100 g) was recorded in carrots whereas the lowest amount of B-carotene (0.034 mg/100 g) was recorded in apple juice. Among the mango verities juices, the highest amount of B-carotene content (1.955 mg/100 g) was recorded in fresh Ao (flim package) followed by Ao mango and xiangya mango juice.

DPPH

DPPH method is a quick method to analyze the free radical activity of natural compounds (Shahzor et al., 2015). The antioxidant activity of a substance can be expressed as its ability to scavenge the DPPH free radical. The DPPH scavenging activity of fresh fruit and vegetable juices is related to phenolic contents. With the increasing IC_{50} value, the decreasing trend of antioxidant activity was found in fruit and vegetable juices and vice-versa (Figure 1). The highest IC_{50} value (0.440 mg/ml) with the lowest antioxidant activity was found in fresh carrot juice as compared to other fruits juices. However, among the mango juices, the highest IC_{50} value (0.032 mg/ml) with the lowest antioxidant activity was recorded in Ao mango juice when compared with the Ao mango (flim package) and Xiangya mango juices.

ABTS

ABTS is a technique to determine the antioxidant activity of hydrogen donating antioxidants and of chain breaking antioxidants and commonly used to estimate the total antioxidant power of single compounds and also in the extracts of different plants (Leong et al., 2002). The ABTS scavenging activity of fresh fruit and vegetable juices are as shown in Figure 1. Among the fresh fruit and vegetable juices, the highest IC_{50} value (0.114 mg/ml) with the lowest antioxidant activity was determined in carrot juice. Among the different mango varieties juices, the highest IC50 value (0.032 mg/ml) with the lowest antioxidant activity was recorded in AO mango juice followed by Ao mango (flim package) and Xiangya mango juices.

Reducing power

The reducing power is an important indicator therefore to determine the potential antioxidant activity. The reducing power of test samples and their actions are usually monitored by the formation of Perl's Prussian blue at 700 nm (Shahzor et al., 2015). The reducing power of fresh fruit and vegetable juices are as shown in Figure 1. The reducing power with the highest EC_{50} value (0.114 mg/ml) and lowest antioxidant activity was recorded in carrot juices as compared to other fruit and vegetable juices. Among the mango varieties juices, the reducing power activity with the highest EC_{50} value (0.032 mg/ml) and lowest antioxidant activity was found in AO mango as compared to Ao mango (film package) and Xiangya mango juice.

DISCUSSION

The attraction of consumers toward food materials have recently increased due to their richness in natural substances. These natural substances are major source for human health (Micha et al., 2017). Antioxidants are one of those natural substances which are involved in inhibition of the oxidation of biomolecules such as lipids, proteins and nucleic acids. Antioxidants have been categorized into two major groups, that is, enzymatic and on-enzymatic antioxidants. Free radical scavenging enzymes such as superoxide dismutase and glutathione reductase are enzymatic antioxidants whereas water soluble compounds such as vitamin C and polyphenol and lipid soluble compounds such as vitamin E and pre-vitamin A are non-enzymatic antioxidants (Barbosa et al., 2017). Numerous redoxactive antioxidants such as polyphenol, tocopherols, carotenoids and ascorbic acids are also found in food materials (Liu et al., 2014). These antioxidants have numerous human health benefits such as they protect from the neurodegeneratives disorders, coronary diseases and cardiovascular diseases (Nemec et al*.,* 2017). High consumption of fruits has been linked due to their refreshing tastes and presence of antioxidants compounds. Apples are significant source of phenolic compounds and are commonly consumed in Europe and North American diets (Hua et al., 2016). Remarkably high antioxidant capacity of guava fruit (*Psidium guajava*) due to the presence of high level of phenolic compounds, therefore occupies a distinct position among tropical fruits (Verma et al., 2018). Musk lime (*Citrus microcarpa*) is commonly used to flavor food and beverages. Moreover, musk lime can also be eaten as fresh fruit. Vitamin C is a major antioxidant ingredient in must melon (Hoyle and Santos, 2010). Mango (*M. indica* L.) being one of most consumed tropic fruit is rich in dietary antioxidants such as ascorbic acid, carotenoids and phenolic compounds. These compounds are involved in the protection of human against various diseases (Barbosa et al., 2017; Liu et al., 2014). Citrus fruits contain wide variety of vitamins and nutrients. For example oranges are rich in compounds such as

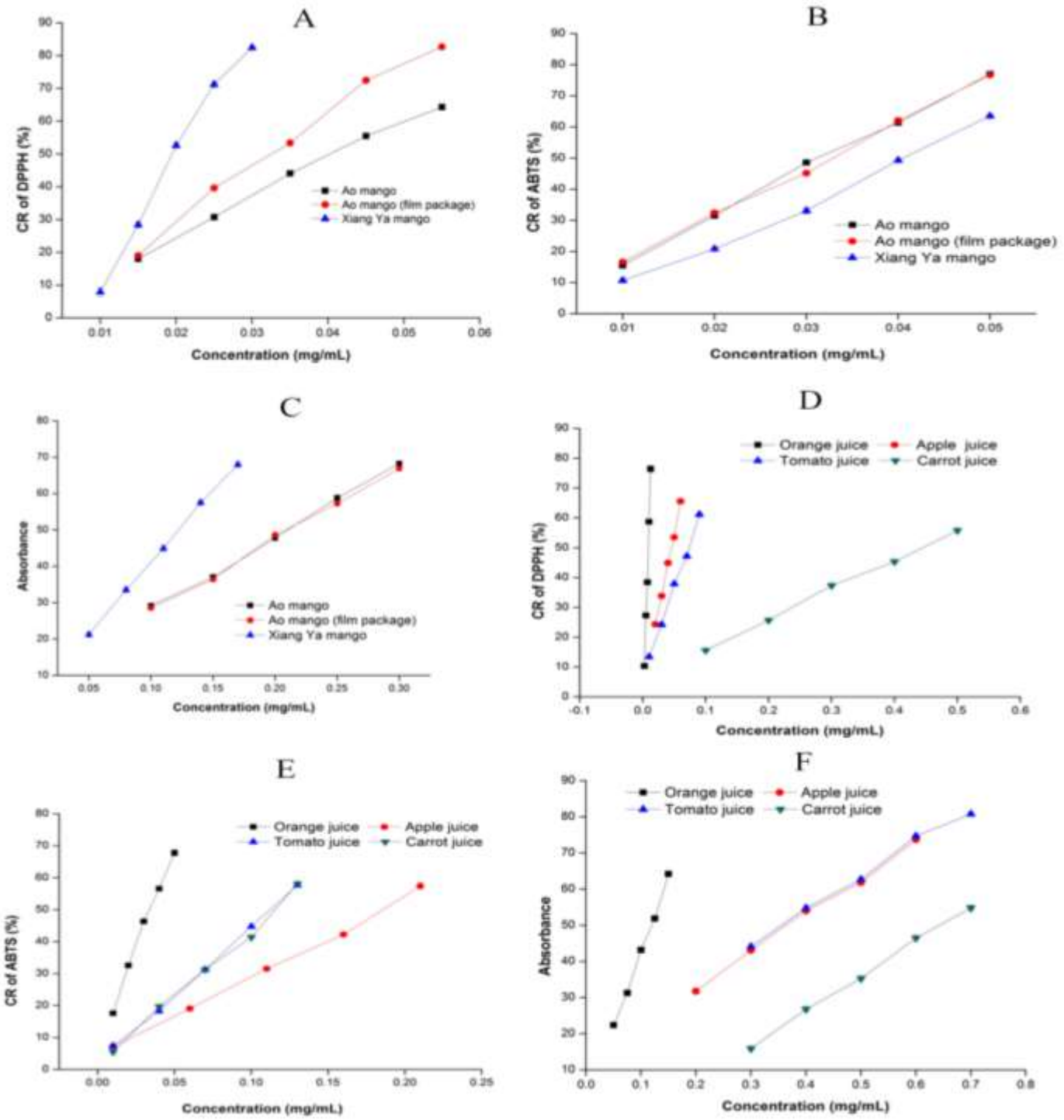

Figure 1. Comparison of DPPH three mangoes (AO, film packaging, Xiangya) (A), Comparison of ABTS of three mangoes (AO, film packaging, Xiangya) (B), Comparison of reducing power of three mangoes (AO, film packaging, Xiangya) (C), Comparison of DPPH of orange, apple, carrot and tomato juices (D), Comparison of ABTS of orange, apple, carrot and tomato juices (E), Reducing power of orange, apple, carrot and tomato juices (F).

limonoids which is bitter in taste and vitamins such as vitamin C. Moreover, oranges also supply carotenoids, folates and phenolic compounds. Since the mentioned oranges are rich in vitamin C, however, the antioxidant activity of orange Juices greatly depends on their phenolic compounds rather than their vitamin contents (Chanalia et al., 2018). In food industries, fruit juices are subjected to evaporation to remove majority of the water

to provide better condition for storage. Transport and preservation of liquid food materials with the lowest handling cost is very important. In addition to evaporation, heating also plays a significant role to increase the phenolic contents through the extra extraction step. Therefore, fruit juice concentrate or commercial fruit juice that reconstitute from the concentrate was believed to be good source of functional food which can easily replace the carbonated soft-drink (Al-Juhaimi and Ghafoor, 2013). Shikmate and phenyl propanois pathways play an important role in the production of phenolic compounds. These phenolic compounds having aromatic ring and bearing one or more hydroxyl groups consists of significant amount of secondary metabolites (Barbosa Gamez et al., 2017). In a previous research, high correlation was found between the content of polyphenols and antioxidant capacity of natural food (Liu et al., 2014). Vitamins such as vitamins C, E and pro-vitamin A have numerous health benefits, however long term clinical studies have revealed that these vitamins cannot reduce the risk of various stress related strokes and cardiovascular diseases. As antioxidants, the phenolic compounds have profound health benefits than vitamins (Nemec et al*.,* 2017). In the present research, the amount of phenolic compounds of both fresh and commercial fruit juices is considerably low, since the daily polyphenol compounds of the diet was estimated to range between 150 mg and 1 g/day (Ribeiro et al., 2007). The daily intake of 100 ml of commercial fruit juice (with 5% or 5 g of fruit concentrate) only contributes to maximum 65 mg of phenolic compounds (13 mg of phenolic compound in 1 g of mango juice). These results revealed that dependence on either fruit or commercial fruit juices does not reflect sufficient daily phenolic compounds intake. In the present study, the fresh apple and guava were blended because peel was previously found to contain additional flavonoids which were not reported in the flesh of fruits (Wolfe et al., 2003). Interesting, these blending method, fail to preserve the antioxidant and phenolic content of fresh apple juices. Among all the fresh fruits the phenolic compounds of fresh apples were comparatively the lowest as compared to all other tested fresh fruit juices. Nevertheless, additional antioxidant capacity may be achieved by keeping peel of the guava. The reason is fresh guava contains the highest amount of polyphenol content. Moreover, the polyphenol contents of fresh guava have potential to inhibit DPPH scavenging activity with the lowest inhibition concentration. Folin-Ciocalteu test showed that the antioxidant activity of the tested fruit juice in DPPH scavenging assay was correlated with the phenolic compounds of fruit juices. This result suggested that phenolic content plays an important role in the antioxidant capacity of various fruits. However, major vitamins (such as vitamin C, vitamin E and pro vitamin A) are not playing significant role in the antioxidant capacity of various fruits. Among the citrus fruits, orange and lime were found to have high amounts of vitamin C contents and phenolic compounds. In the present research the antioxidant capacity of both fresh orange and lime juices were found to be similar in both phenolic quantification and DPPH scavenging test. This result suggested that both citrus fruits may contain similar bioactive compounds which play role in their antioxidant capacity. The decline in the antioxidant activity in commercial fruit juice might be due to long term storage of raw fruit or fruit concentrate before the production and packaging of ready to drink fruit juice. Increase of oxygen, pH and temperature during storage of fruits are involved in the reduction of antioxidant activity of the fruit concentrates (Moon et al., 2018). Moreover, short term *in-vivo* consumption of orange juice lost the potential to affect the oxidation stress that is related to cardiovascular risk (Jae et al., 2014). The reason could be inadequate amount of daily phenolic compounds intake since the phenolic compounds concentration was significantly low. The whitening effect of all of the freeze dried fruit juices is also linked to antioxidant activity. The present research clearly revealed the potential value of fruit juices which are substitute for fresh fruit. The study further revealed that among the various fruit tested each fruit has different level of antioxidant capacity and total phenolic contents. Commercially produced orange and mango juices contain significant amount of phenolic content and antioxidant capacity as compared to fresh mango and orange blend. Therefore, fresh fruit juices can be considered a good source of natural antioxidants besides fresh fruit. However, fresh fruit still contain much higher phenolic content and antioxidant capacity. From the present study, it can be concluded that fruit juices though expensive than the fresh fruits however should be considered as the secondary choice of dietary fruits when fresh fruits are not reachable.

Conclusion

Fresh produced orange and mango juices contain significant amount of phenolic content and antioxidant capacity as compared to fresh mango and orange blend. Therefore, fresh fruit juices can be considered a good source of natural antioxidants besides fresh fruit. However, fresh fruit still contain much higher phenolic content and antioxidant capacity. From the present study, it can be concluded that fruit juices though expensive than the fresh fruits however should be considered as the secondary choice of dietary fruits when fresh fruits are not reachable.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Afifa K, Kamruzzaman M, Mahfuza I, Afzal H, Arzina H, Roksana HA (2014). Comparison with antioxidant and functional properties among five mangos (*Mangifera indica* L.) Varieties in Bangladesh. International Food Research Journal 21:1501-1506.

Ahmed SM, Luo L, Namani A, Wang XJ, Tang X (2017). Nrf2 signaling pathway Pivotal roles in inflammation. Biochimica et Biophysica Acta 1863:585-597.

Al-Juhaimi F, Ghafoor K (2013). Bioactive compounds, antioxidant and physico-chemical properties of juice from lemon, mandarin and orange fruits cultivated in Saudi Arabia. Pakistan Journal of Botany 45:1193-1196.

Barbosa GI, Caballero MKP, Ledesma N, Sayago ASG, Garcia MML, Bishopvon WEJ, Montalvo GE (2017). Changes in the nutritional quality of five Mangifera species harvested at two maturity stages. Journal of Science of Food and Agriculture 97(14):4987-4994.

Cabral DOA, Barros VI, Silva CA, Bechara EJH, Paes DB (2009). Total phenolic content and free radical scavenging activities of methanolic extract powders of tropical fruit residues. Food Chemistry 115:469-475.

Carlisi D, Anneo A, Martinez R, Emanuele S, Buttitta G, Fiore R, Vento R, Tesoriere G, Lauricella M (2014).The oxygen radicals involved in the toxicity induced by parthenolide in MDA-MB-231 cells. Oncology Reports 32:167-172.

Chanalia P, Gandhi D, Anjana Bala DS Singh J (2018) Antioxidant activity and nutritional value of Citrus limetta and Ananas comosus pomace. Journal of Food Science and Nutrition 4(1):004-007.

Dashman T, Blocker DE, Baker N (1996). Laboratory manual for human nutrition 2ed New York NY: Harwood Academic Publishers.

Heo H, Kim Y, Chung D, Kim D (2007). Antioxidant capacity of individual and combined phenolics in a model system. Food Chemistry 104:87-92.

Hoyle CHV, Santos JH (2010). Cyclic voltammetric analysis of antioxidants activity in citrus fruits from Southeast Asia. International Food Research Journal 17:937-946.

Hua Z, Rong T (2016). Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. Current Opinion in Food Science 8:33-42.

Hymavathi K, Hymavathi TV, Khader V (2005). Carotene, ascorbic acid and sugar content of vacuum dehydrated ripe mango powders stored in flexible packaging material. Journal of Food Composition and Analysis 18:181-192.

Jae HP, Minhee L, Eunju P (2014). Antioxidant Activity of Orange Flesh and Peel Extracted with Various Solvents. Preventive Nutrition and Food Science 19(4):291-298.

Lauricella M, Carlisi D, Giuliano M, Calvaruso G, Cernigliaro C, Vento R, Anneo A (2016). The analysis of estrogen receptor- positive breast cancer stem-like cells unveils a high expression of the serpin proteinaseinhibitor PI-9: Possible regulatory mechanisms. International Journal of Oncology 49:352-360.

Lee Y, Hwang KT (2017). Changes in physicochemical properties of mulberry fruits (*Morus alba* L.) during ripening. Scientia Horticulturae 217:189-196.

Lemmens L, Tchuenche ES, Loey AMV, Hendrickx ME (2013). Beta-carotene isomerization in mango puree as influenced by thermal processing and high-pressure homogenization. European Food Research Technology 236:155-163.

Leong LP, Shui G (2002). An investigation of antioxidant capacity of fruits in Singapore markets. Food chemistry 76(1):69-75.

Lidia M, Victor DF, Paula B, Marica C (2011). Comparative antihemolytic and radical scavenging activates of strawberry tree (*Arbutus unedo* L.) Leaf and fruits. Food and Chemical Toxicology 49:2285-2291.

Liu F, Li R, Wang Y, Bi X, Liao X (2014). Effects of high hydrostatic pressure and high-temperature short-time on mango nectars: Changes in microorganisms, acid invertase, 5-hydroxymethylfurfural, sugars, viscosity, and cloud. Innovative Food Science and Emerging Technologies 22:22-30.

Mendes L, Freitas VD, Baptista P, Carvalho M (2011). Comparative antihemolytic and radicalscavenging activities of strawberry tree (*Arbutus unedo* L.) leaf and fruit. Food and Chemical Toxicology 49:2285-3229.

Micha R, Peñalvo JL, Cudhea F, Imamura F, Rehm CD, Mozaffarian D (2017). Association between Dietary Factors and Mortality from Heart Disease, Stroke, and Type 2 Diabetes in the United States. The Journal of the American Medical Association 317:912-924.

Moon HC, Han GJ, Min JK, Min JK, Hyun JS (2018). Fruit Juice Supplementation Alters Human Skin Antioxidant Levels *In Vivo*. Case Study of Korean Adults by Resonance Raman Spectroscopy Biotechnology and Bioprocess Engineering 1:116-112.

Musa KH, Abdullah A, Jusoh K, Subramaniam V (2011). Antioxidant activity of pink flesh guava (*Psidium guajava* L.). Effect of extraction techniques and solvents Food Analytical Methods 4:100-107.

Natal D, Rodrigues K, Moreira M (2017). Bioactive compounds of the Ubá mango juices decrease inflammation and hepatic steatosis in obese Wistar rats. Journal of Functional Foods 32:409-418.

Nemec MJ, Kim H, Marciante AB, Barnes RC, Hendrick ED, Bisson WH, Talcott ST, Mertens-Talcott SU (2017). Polyphenolics from mango (*Mangifera indica* L.) suppress breast cancer ductal carcinoma in situ proliferation through activation of AMPK pathway and suppression of mTOR in athymic nude mice. Journal of Nutritional Biochemistry 41:12-19.

Perera CO, Yen GM (2007). Functional properties of carotenoids in human health. International Journal of Food Properties 10: 201-230.

Ranganna S (1999). Hand book of Analysis and quality control for Fruits and Vegetables Products. Second Edn Tata McGraw-Hill Publishing Company Limited New Delhi.

Re R, Pellegrini N, Proteggente A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cationdecolorization assay. Free Radical Biology and Medicine 26:1231-7.

Ribeiro SM, Queiroz JH, Queiroz M, Campos F, Pibeiro S (2007). Antioxidants in mango (*Mangifera indica* L.) pulp. Plant Foods for Human Nutrition 62:13-17.

Shahzor GK, Ying L, Aijaz HS, XI F, Marry BS, Yan FW, Wen H (2015). Characterization of Auricularia auricular polysaccharides and its antioxidant properties in fresh and pickled product. Journal of Biological Systems 81:387-395.

Sluijs I, Cadier E, Beulens JWJ, Van-der ADL, Spijkerman AM, Van-der SYT (2015). Dietary intake of Carotenoids and risk of type 2 diabetes. Nutrition, Metabolism, and C cardiovascular Diseases 25:376-381.

Ulla A, Rahman MT, Habib ZF, Rahman MM, Subhan N, Sikder B, Reza HM, Hossain MH, Alam MA (2017). Mango peel powder supplementation prevents oxidative stress, inflammation, and fibrosis in carbon tetrachloride induced hepatic dysfunction in rats. Journal of Food Biochemistry 41:12344.

Verma M, Rai GK, Kaur D (2018). Effect of extraction solvents on phenolic content and antioxidant activities of Indian gooseberry and guava International Food Research Journal 25(2):762-768

Viuda MM, Ruiz NY, Fernández LJ, Sendra E, Sayas BE, Pérez ÁJA (2011). Antioxidant properties of pomegranate (*Punicagranatum* L.) bagasses obtained as co-product in the juice extraction. Food Research International 44:1217-1223.

Wolfe K, Wu X, Liu RH (2003). Antioxidant activity of apple peels. Journal of Agricultural and Food Chemistry 51:609-614.

Yeap SK, Ho WY, Beh BK, Liang WS, Ky H, Yousr AHN, Alitheen NB (2010). Vernonia amygdalina, an ethnoveterinary and ethnomedical used green vegetable with multiple bioactivities. Journal of Medicinal Plants Research 4:2787-2812.

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Evaluation and differential expression of genes conferring drought tolerance in selected maize genotypes in the Morogoro Region of Tanzania

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Experiments were conducted at Sokoine University of Agriculture in Morogoro, Tanzania to assess the growth performance and grain yield of ten maize cultivars under well-watered and water stressed conditions. The ten cultivars (CML 444, CML395, CML539, WE 4107, WE 2112, WE 3102, WE 4112 and WE 4116) obtained from Water Efficiency Maize for Africa (WEMA) project and two local market cultivars ('STAHA' and 'TMV1') were grown in a Randomized Complete Block design with a 10×3 factorial treatment arrangement and three replications. The three drought stress treatments were 50, 75 or 100% of field capacity with 10 kpa (equivalent to field capacity) and 30 kpa (drought) using tensiometers. Drought stress was initiated at vegetative stage (three weeks after emergence) for thirty days and flowering stage (one week before to two weeks after flowering). Plant height, stem diameter, leaf length, leaf width and chlorophyll content were measured at weekly intervals from two weeks after planting to maturity. Days to anthesis and silking were used to calculate Anthesis-Silking Interval (ASI) and kernel dry mass were recorded at harvest. Vegetative growth responses were not affected by water stress, but plant height and leaf chlorophyll content tended to decrease, while ASI ranging from 5.2 to 11.1 days and kernel dry mass decreased with increased drought stress. Dry kernel weight was significantly greater among five of the cultivars designated drought tolerant and the lowest in the two drought susceptible cultivars. Drought tolerant genes were expressed at different levels and only a few cultivars expressed all three genes at the vegetative and flowering stages. Thus, vegetative response of maize to drought stress varied based on cultivars. However, it appears that drought stress exerted more impacts on reproductive processes compared to vegetative.

Key words: Cultivars, drought, genotypes, grain yield, growth performance, hybrids.

INTRODUCTION

Maize (*Zea mays* L.) is commonly grown worldwide and used as food (animals and humans) and as transportation fuel in developed countries. In Tanzania, maize is the main staple as well as cash crop and is produced on approximately 4.0 million ha in almost all of the four official agro ecological zones by small or limited resource

farmers compared to rice, wheat, millet and sorghum and is their main source of income (DTMA, 2014). Maize contributes about 60 and 30% of dietary and utilizable protein, respectively (DMTA, 2014; Lyimo, et al., 2001).

Despite the increase in land acreage, production is still very low averaging 1.37 t ha^{-1} grain yield due to limited moisture, while 5 t ha^{-1} are required to satisfy the food demand (DTMA, 2014). Due to a fast growing population, it is estimated that maize production must increase by 3.0 to 3.5% annually to satisfy demand, which exceeded the 2.0% growth rate over the past two decades (Lyimo et al., 2001).

Climate change has caused a disruption in rainfall patterns and thus no longer a reliable source of moisture especially at flowering, pollination and embryo development stages to produce a harvestable crop. For example, most of the agro ecological regions (Magehema et al., 2014), that receive rains twice per year are now either receiving it once or only for short periods despite high maize production. Maize requires high amounts of moisture to produce a complete crop especially at tasseling, silking and blister kernel stages (Xu et al., 2014). The southern highlands receive the highest percent distribution in terms of area and amount of maize production of 26%, followed by Lake, Eastern, Northern, Western, Southern and Central zones of 25, 13, 12, 10, 8 and 6%, respectively (DMTA, 2014).

Drought is the most limiting factor for maize production especially if deficit occurs at grain filling and dough stages and is first among the criteria considered by farmers when selecting varieties (Lyimo et al., 2001). In maize production, moisture requirements fluctuate based on growth stages with greatest requirements. There are few drought tolerant maize varieties in the country that were developed through other on-going projects. These varieties have been used in some ecological zones although their genetic diversity is not well known.

Drought stress greatly reduces net photosynthesis rates in plants (Mafakheri et al., 2010), and as the intensity of drought stress increases, the photosynthetic rate decreases even further, eventually reducing yield (Liu et al., 2012). The synthesis of chlorophyll a and b is inhibited as well as a reduction in the content of chlorophyll a and b binding proteins, leading to a decline in the light-harvesting pigment proteins associated with photosystem II (Farooq et al., 2009). Plants under water deficit reduce transpirational water losses by reducing stomatal conductance causing partial or complete closure (Ghannoum, 2008). Stomata closure reduces CO_2 uptake thus reducing photosynthetic rates, transpirational cooling, water and nutrient uptake (Lu et al., 2011).

Kamara et al. (2003) revealed that water deficit imposed at various developmental stages of maize reduced total biomass accumulation at silking by 37%, at grain filling by 34% and at maturity by 21%. Therefore, moisture stress can cause delay in silk emergence, hence increasing the anthesis-silking interval (ASI; the time in days between pollen shed and silk emergence) (Aylor et al., 2003) or silks may desiccate and become non-receptive to pollen germination.

Delay in silk emergence is connected to a delay in silk elongation, a process, which takes place through cell division and expansion. Water deficit affects cell expansion, which minimizes silk elongation rates, sometimes to zero, which in turn affects ASI (Monneveux et al., 2006). Longer ASI causes asynchronies in maize flowering that result in incomplete pollination and kernel set, hence yielding losses. The first two weeks after silking are most critical for fertilization and embryo development both of which require high amounts of moisture. Water stress at this stage causes embryo abortion which in turn affects final grain yield (Hayano-Kanashiro et al., 2009; Wei et al., 2009), reduces silk length, inhibits embryo development after pollination and reduces ear size and number of kernels to be fertilized (Farooq et al., 2009).

There are a set of drought tolerant genes that are known to control physiological and biochemical processes of plants during water stress and breeding practices such as inbreeding or breeding open pollinated varieties (OPVs) reduce genetic diversity among plant materials. Current research shows that most of maize varieties developed in Africa are OPVs and the genetic features of most of them are not known.

The objectives of this study were (1) to compare water use, grain yield and growth responses of ten maize cultivars under well-watered and water stressed conditions and (2) to assess the expression of drought tolerant genes at both vegetative and flowering stage to identify tolerance or susceptibility.

MATERIALS AND METHODS

Greenhouse experiments were conducted in a Randomized Complete Block Design with a 10×3 factorial treatment arrangement and three replications at Sokoine University of Agriculture (SUA) in Morogoro, Tanzania. The treatment combinations were moisture at 100 (equivalent to field capacity), 75 and 50%, respectively, of field capacity and ten maize hybrid varieties eight of which ('CML444', 'CML395', 'CML539', 'WE4107', 'WE2112', 'WE3102', 'WE4112' and WE 4116) were obtained from the Water Efficiency Maize for Africa (WEMA) project located at the Ilonga Agricultural Research Institute-Morogoro, Tanzania and are classified as being drought tolerant. The remaining two hybrid maize hybrid varieties, STAHA and TMV1, are regularly grown by local farmers and are classified

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as being drought susceptible.

Planting and treatment initiation

The oven dry weight method was used to determine percent soil moisture that approximates field capacity, beginning with determining the gravimetric moisture content (Gardner, 1986). A 1000 ml graduated cylinder with a hole drilled in the bottom, to allow air to escape when water was added was filled with a random subsample of air-dried soil, which was leveled to a similar consistency. The surface of the soil was covered with paper towel and 100 ml of water were poured slowly onto the surface to obtain an even distribution through the column. The cylinder was covered to avoid evaporation and allowed to equilibrate stand for 24 h following which there was a sharp line where the water stopped moving in the soil column. Triplicate soil samples were collected from the cylinder about 5 cm above the wetting front and dried at 100°C to a constant weight after which dry weight was recorded and gravimetric moisture fraction was determined using the fresh to dry weight ratio. This information was used to determine the amount of oven-dry soil per pot, and watering to field capacity (1 L) was done by weighing the pots.

Ten liters pots were filled with 10 kg of air-dried soil into which diammonium phosphate ($(NH_4)_2HPO_4$; 18-46-0) fertilizer was thoroughly incorporated based on soil test recommendations for the Morogoro region. The drought stress treatments of 100 (field capacity), 75 and 50% of field capacity were initiated at vegetative stage (three weeks after emergence) for thirty days watered again for one week, and then imposed one week before flowering stage to two weeks after. Field capacity moisture (well-watered) was kept at 10 kpa and drought stress kept at 30 kpa using tensiometers.

Phenological measurements taken included plant height, stem diameter, leaf length, width and chlorophyll content. Leaf chlorophyll content was measured for fully expanded leaves using an Apogee CCM 200+ chlorophyll content meter (Apogee Instruments, Logan UT, USA) beginning one week after emergence to harvest, while leaf length and width were assessed on one leaf per plant up to maximum growth (no further increases in either length or width), corresponding to about six to eight weeks after emergence.

Gene expression profiling

Mature disease free maize leaves were collected at the early vegetative and flowering stages of plant development from all ten genotypes cleaned with RNAlater and stored at -80°C. RNA was isolated according to the CTAB method (Hilario and MacKay, 2007). A 0.3 g leaf sample was ground manually using a mortar and pestle in 300 µl of buffer followed by an additional 700 µl and further ground and then centrifuged at 12000 g for 5 min at 4°C. The supernatant was transferred to new Eppendorf tubes, containing 200 µl of acid water to which extracted chloroform was added and shaken vigorously for 15 s and incubated at room temperature for 3 min. The sample was centrifuged at 12000 g for 15 min at 4°C, and the upper aqueous phase transferred to a new tube followed by the addition of 0.5 ml of isopropanol, mixed, and incubated at room temperature for 10 min, and centrifuged at 12000 g for 10 min at 4°C. The supernatant was discarded and 1.5 ml of 75% ethanol was added to the pellet, vortexed briefly and centrifuged at 12000 g for 5 min at 4°C. The supernatant was discarded and the pellets allowed to dry after which they were dissolved in 20 µl of RNase free water, briefly centrifuged and then transferred to a new tube. The quality and quantity of total RNA was checked using Nano drop and native 1% agarose gel in TBE.

Riverse transcriptase polymerase chain reaction (RT-PCR) and real time PCR (qPCR)

Total cDNA was synthesized from total RNA utilizing NotI-OligodT bifunctional primer with Access Quick RT-PCR system (Promega Corp., Fitchburg WI) as per the manufacture's guidelines. Total cDNA was utilized in downstream quantitative expression profilings in RT-PCR with SYBR-Green. Primers utilized for expression profiling included *ProDH, UBQ7, Ubiquitin, P5CS, P5CR* and *Maize actin (internal reference)*. The conditions for amplifications were an initial denaturation at 95°C for 2 min, a final denaturation at 95°Cfor 30 s, annealing at 53°C for 30 s for (*ProDH*) and 50°C for other primers, extension at 72°C for 30 s, a final extension at 72°C for 5 min and a 4°C hold. Of the five primers, only *ProDH, UBQ*7 and *Ubiquitin* showed good amplification bands and therefore they were used for further qPCR analysis. Quantitative qPCR was performed using a Thermo scientific maxima SYBR Green/Fluorescein qPCR master mix (2×) as per the manufacturer's instructions.

RESULTS AND DISCUSSION

Biomass production

The analysis of variance to cultivar, drought stress, and the cultivar×drought stress interactions for plant height, stem diameter, leaf length and width, chlorophyll content, anthesis-silking interval (ASI) and dry seed weight of the ten maize varieties are shown in Table 1. Drought stress had no significant impact on plant height or stem diameter, but enhanced chlorophyll content, ASI and weight of dry kernels, while cultivar significantly influenced all variables.

While the main effect of drought stress on plant height was not significant, certain trends were evident. Plant height and leaf chlorophyll content tended to decrease with increased water stress while ASI and dry seed weight decreased significantly with increased drought stress (Table 2).

For cultivar main effects, hybrids 'TMV1' and 'STAHA' responded differently to drought stress as compared to the drought tolerant hybrids (Table 3). 'STAHA' had bigger stems than all other cultivars studied and leaf chlorophyll concentration was similar for 'STAHA', 'TMV1', 'WE4116', 'WE4107' and 'WE210'.

'TMV1' and 'STAHA', the regular hybrids on the market were decidedly taller than the other cultivars while the other eight cultivars had similar heights (Table 3). The ASI ranged from 5.2 days to 11.1 days, with 'CML444' and 'WE4107' having the shortest while, 'WE4116' and 'WE4107' had the longest but similar to that of 'CML539', 'WE3102', 'WE4112', 'TMV1' and 'STAHA'. Dry seed weight was significantly greater among five of the cultivars designated drought tolerant and the lowest in the two drought susceptible cultivars.

Gene expression profiling

For expression levels determined at the vegetative stage (Figure 1), *ProDH* gene was expressed significantly in all

Table 1. Statistical significance from analysis of variance (ANOVA) of cultivar, drought, time (weeks) and their respective interactions for plant height, stem diameter, chlorophyll content and dry seed weight of maize (*Zea mays* L.).

Source of variation[z]	df	Plant diameter (cm)	Stem diameter (cm)	Chlorophyll content ($\mu molm^{-2}$)	Anthesis silking interval (days)	Dry seed weight
Drought (DR)	2	NS	NS	*	***	**
Cultivar	9	***	***	***	***	*
Cultivar*DR	18	NS	NS	NS	NS	*

All biomass and phenological data are the mean of three replications of one plant each. Plant height and stem diameter are expressed in centimeters (cm), chlorophyll content as micro mole per square meter per second, and dry seed weight as grams per plant. Anthesis silking interval is the number of days between the appearance of the tassel and the emergence of the silk ans is expressed in days. [z]*,**,*** Significant at 0.05, 0.01, and 0.001 levels of probability, respectively.

Table 2. The main effect of drought stress on plant height, chlorophyll content, anthesis-silking interval (ASI), plant height and dry seed weight of ten maize cultivars. [z]

Drought plant stress (%)	Plant height (cm)	Chlorophyll content ($\mu mol\ m^{-2}$)	Anthesis silking interval (days)	Dry seed weight ($g\ plant^{-1}$))
50[x]	76.8^{a}	19.9^{ab}	11.4^{a}	13.3^{b}
75	78.6^{a}	19.6^{b}	9.2^{b}	13.3^{b}
100	80.7^{a}	21.1^{a}	6.7^{c}	29.5^{a}

All biomass and phenological data are the mean of three replications of one plant each. Plant height and stem diameter are expressed in centimeters (cm), chlorophyll content as micro mole per square meter per second, and dry seed weight as grams per plant. Anthesis silking interval is the number of days between the appearance of the tassel and the emergence of the silk ans is expressed in days. [z]Means within a column followed by the same letter are not significantly different (LSD P<0.05).

Table 3. The main effect of cultivar on plant height, stem diameter, anthesis silking interval (ASI), chlorophyll content and total seed weight of ten maize cultivars subjected to drought stress. [z]

Cultivar	Plant height (cm)	Height diameter (cm)	Anthesis-silking interval (days)	Chlorophyll content ($\mu molm^{-2}$)	Dry kernel weight ($g\ plant^{-1}$)
CML 444[x]	75.1^{cd}	3.8^{cd}	5.2^{c}	19.6^{cde}	24.2^{ab}
CML 395	68.7^{de}	4.3^{b}	7.3^{bc}	20.1^{bcd}	26.2^{a}
CML 539	58.9^{e}	3.2^{e}	8.4^{abc}	18.4^{de}	17.3^{b}
WE 4107	82.5^{bc}	3.8^{cd}	11.1^{a}	21.1^{abc}	29.6^{a}
WE 2112	78.8^{cd}	3.7^{d}	8.0^{bc}	21.3^{abc}	27.5^{a}
WE 3102	74.6^{cd}	3.8^{cd}	8.3^{abc}	18.8^{cde}	27.2^{a}
WE 4112	77.1^{cd}	3.4^{de}	10.2^{ab}	17.5^{e}	3.9^{c}
WE 4116	81.3^{bc}	4.2^{bc}	11.1^{a}	20.8^{abcd}	26.6^{a}
TMV1	91.9^{ab}	4.6^{b}	10.9^{ab}	21.4^{ab}	4.8^{c}
STAHA	98.3^{a}	5.2^{a}	10.7^{ab}	23.1^{a}	6.8^{c}

[x]All biomass and phenological data are the mean of three replications of one plant each. Plant height and stem diameter are expressed in centimeters (cm), chlorophyll content as micro mole per square meter per second, and dry seed weight as grams per plant. Anthesis silking interval is the number of days between the appearance of the tassel and the emergence of the silk ans is expressed in days. [z]Means within a column followed by the same letter are not significantly different (LSD P<0.05).

cultivars except in 'STAHA' (J). The highest expression was observed in 'CML395' (B) and 'CML444' (A) and the lowest in 'TMV1' (I). The *UBQ7* gene was expressed practically in all cultivars except in 'STAHA'. The highest *UBQ7* gene expression was observed in 'WE4116' (H) and 'WE2112' (E), while the lowest expression was in'WE3102' (F). The *Ubiquitin* gene was highly expressed in 'WE3102' (F) and lower in 'CML395'.

At flowering, *UBQ7* expression was significantly higher in all cultivars except in 'STAHA (J) that had no

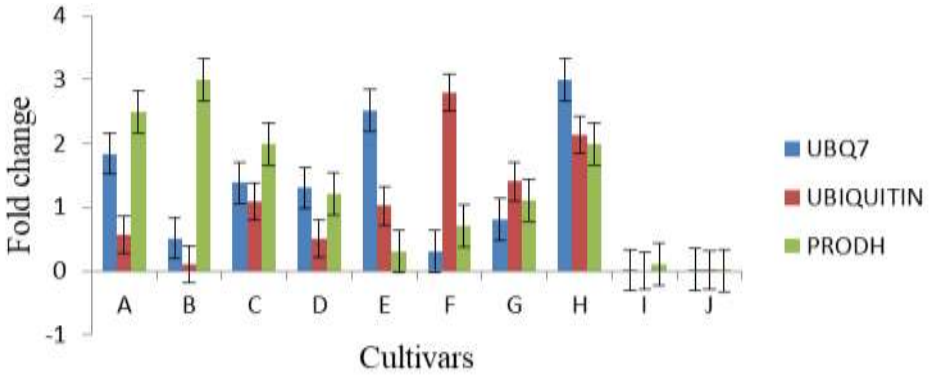

Figure 1. Differential expression of ubq7, ubiquitin and prodh genes in ten maize varieties sampled at vegetative (V6) stage. The relative quantification of gene expression is based on $2^{-\Delta\Delta CT}$ method using maize actin as a reference gene. [z]A-H represents cultivars 'CML444', 'CML395', 'CML539', 'WE4107', 'WE2112', 'WE3102', 'WE4112', 'WE 4116, ''STAHA' and 'TMV1', respectively.

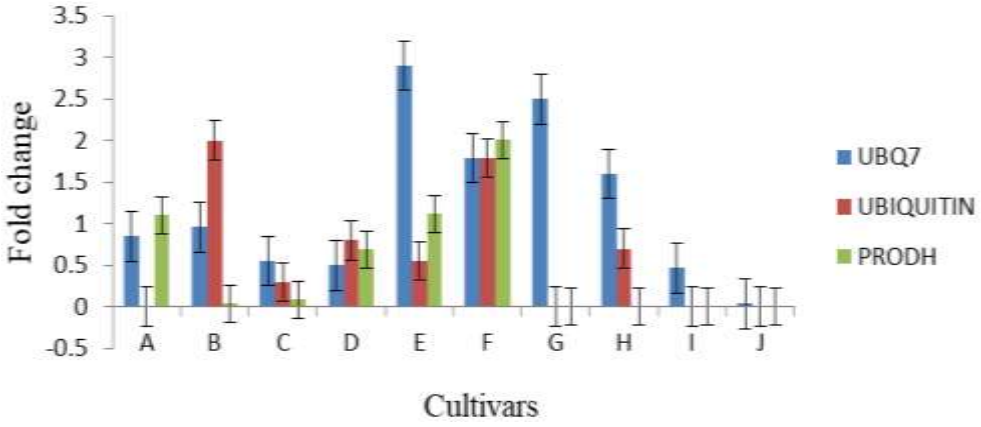

Figure 2. Differential expression of ubq7, ubiquitin and prodh genes on ten maize varieties sampled at flowering stage. The relative quantification of gene expression is based on $2^{-\Delta\Delta CT}$ method using maize actin as a reference gene. [z]A-H represents cultivars 'CML444', 'CML395', 'CML539', 'WE4107', 'WE2112', 'WE3102', 'WE4112', 'WE 4116, ''STAHA' and 'TMV1', respectively.

significant expression of any gene (Figure 2). *Ubiquitin* was not expressed in cultivars 'CML444' (A), 'WE4112' (G), 'TMV1' (I) or 'STAHA' (J), respectively. *ProDH* was also expressed in few cultivars, the highest expression being in 'WE3102' (F) with a fold change of 2.5. All three genes were significantly expressed in cultivars 'CML539'(C), 'WE4107' (D), 'WE2112' (E), and WE3102 (F), while cultivars 'CML395' (B) and 'WE4116' (H) expressed *Ubiquitin* and UBQ7, 'CML444' (A) expressed UBQ7 and *ProDH*, 'WE4112' (G) and 'TMV1' (I) expressed *UBQ7* only.

These results showed that the response of maize genotypes to drought is cultivar dependent. Water stress reduced plant height and leaf width as plants under well-watered conditions were decidedly taller with broader leaves than water stressed plants and the plant height decreased with the increase in water stress. Lack of adequate moisture reduces growth in the meristematic tissue hence a slow rate of plant elongation (Hussain et al., 2008). The reduction in leaf width can also impact photosynthetic activity as a reduction in leaf area means that there is less surface area to trap light for photosynthesis. The opposite was true for stem diameter (stem expansion) as with a higher rate of water stress, plant expansion was not significantly affected, as was leaf length. Chlorophyll content varied with cultivar and tended to be higher under reduced drought stress. Hybrids cultivars 'TMV1' and 'STAHA' responded differently to drought stress as compared to the drought tolerant hybrids.

Drought stress did not reduce any of the traits studied at the vegetative stage since less moisture is required at

this stage but more variation was observed among cultivars rather than among the levels of stress. Drought stress caused an increase ASI in that a shorter ASI was observed with well-watered plants and a longer ASI at 50% drought stress. The average grain yield for drought tolerant genotypes was higher despite the longer ASI of some cultivars than the regular hybrids, which are drought susceptible.

Stress during the pollination and silking period often reduces yield potential of maize and ASI is one of the best indicators. According to Avan et al. (2008), silk development consists of four phases: (1) cell division and tissue expansion occurred together uniformly all along the silk; (2) cell division progressively ceases from tip to base, while expansion remains spatially uniform including during the phase, (3) after the cessation of cell division, and (4) as the silk emerges from the husks, expansion ceases in the emerged portion, probably because of direct evaporative demand, while the relative growth rate progressively decreased in the enclosed part. They indicated that water deficits can reduce the rate of tissue expansion and cell division resulting in delayed silk emergence. This could partly explain the longer ASI observed under drought stress in our study, especially in phase 3 of development, the duration of the time between the end of cell division and the arrest of cell growth in silk apex, corresponded to the anthesis-silking interval used by breeders to characterize the response of cultivars to stress.

Drought tolerant genes are expressed to enable metabolic activities of the plant in order to complete its life cycle when there is water stress. Maize requires more water at flowering through the grain filling stage than at any other growth stage (Kranz et al., 2008). Drought stress in this study was imposed at vegetative (V6) stage and at flowering (tasseling to silking stage). At flowering, the *UBQ7* gene was up regulated in cultivars 'CML395', 'WE2112', 'WE3102', 'WE4112' and 'STAHA', *ubiquitin* gene was up regulated in cultivars 'CML395' and 'WE4107', while *ProDH* gene was up regulated in cultivars 'WE2112' and 'WE3102'. Therefore, cultivars with genes up regulated at flowering may be considered to have higher tolerance levels as compared to those that were down regulated although this inference would have to be confirmed in further studies, in which a greater number of drought tolerant genes may be considered and in which plants may be subjected to greater levels of stress.

At vegetative stage, all genes studied were highly expressed although expression varied with cultivars. Gene expression at flowering showed a down regulation of some genes that were highly expressed at the vegetative stage. The down regulation of drought tolerant genes probably affects photosynthesis (Photosystems I and II) and on energy metabolism processes both at vegetative and reproductive stages (Batlang et al., 2014).

Of the ten cultivars, only in three, namely 'WE4107', 'WE3102' and 'WE2112', was expression of these genes high both at vegetative and flowering stages. This suggests that these cultivars may be considered more drought tolerant than others in this study.

Genetic diversity determinations showed that cultivars 'WE4107' and 'WE2112' were quite similar with a similarity coefficient of 1, while 'WE3102' was marginally different from the other two with a similarity coefficient of 0.91 on both cultivars.

Conclusion

Drought tolerant and susceptible maize cultivars were used to assess growth, development and grain yield, drought gene expression level and genetic diversity. Drought had no significant effect on vegetative growth but exerted significant impacts on ASI and grain yield. Drought tolerant genes were expressed at different levels and only a few cultivars expressed all three genes at the vegetative and flowering stages. These genes were mostly down regulated at flowering than at the vegetative stage. Therefore, based on a short ASI, high dry mass of kernels and drought gene expression at both vegetative and flowering stages, cultivars 'WE2112', 'WE3102' and 'WE4107' appeared to be tolerant of the drought stress levels evaluated in this study. Thus, these cultivars which have shown a degree of drought tolerance, could be tested again through on-farm field trials in regions of the country where drought is severe, followed by differential gene expression using many other drought tolerant genes which were not involved in this study, and could form the basis for developing other drought tolerant varieties.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Avan AH, Tardieu F, Turc O (2008). Drought-induced changes in anthesis-silking interval are related to silk expansion: a spatio-temporal growth analysis in maize plants subjected to soil water deficits. Plant, Cell and Environment 31:1349-1361.

Aylor DE, Schultes NP, Shields EJ (2003). An aerobiological framework for assessing cross-pollination in maize. Agriculture Forest Meteorology 119:111-129.

Batlang U, Madana MR, Ambavaram A, Pereira A (2014). Drought responsive genes and their functional terms identified by GS FLX Pyrosequencing in maize. Maydica Electronic Publication. Kindly Mydica 59:306-314.

Drought-tolerant maize for Africa (DTMA) (2014). A quarterly bulletin of the drought tolerant maize for Africa project. http://dtma.cimmyt.org/index.php/publications/doc_details/188-dt-maize-a-quarterly-bulletin-of-the-drought-tolerant-maize-for-africa-project

Farooq M, Wahid A, Kabayoshi N, Fujita D, Basra SMA (2009). Plant drought stress: effects, mechanisms and management. Agronomy for sustainable development, Springer Verlag (Germany) 29(1):185-212.

Gardner WH (1986). Water content. Chap. 21 in Klute, A., ed. Methods of soil analysis. Part 1. Physical and mineralogical methods. 2nd Ed. Soil Science Society of America, Inc. Madison, Wisconsin.

Ghannoum O (2008). C_4 photosynthesis and water stress. Annals Botany doi:10.1093/aob/mcn093, http://www.aob.oxfordjournal.org. Retrieved December 28, 2014.

Hayano-Kanashiro C, Calderon-Vazquez C, Ibarra-Laclette E, Herrera-Estrella L, Simpson J (2009). Analysis of gene expression and physiological responses in three mexican landraces under drought stress and recovery irrigation. PLoS One 4:1-19.

Hilario MM, MacKay JF (2007). Protocol for nucleic acid analysis by no-radioactive probes 2nd edition. Humana Press Inc. New York, NY.

Hussain M, Malik MA, Farooq M, Ashraf MY, Cheema MA (2008). Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. Journal Agronomy Crop Science 194:193-199.

Kamara AY, Menkir A, Badu-apraku B, Ibikunle O (2003). The influence of drought stress on growth, yield and yield components of selected maize genotypes. Journal Agricultural Science 141:43-50.

Kranz WL, Irmak S, van Donk SJ, Yonts CD, Martin DL (2008). Irrigation management for corn. NebGuide G1850. University of Nebraska-Lincoln Extension. University of Nebraska- Lincoln Extension Bulletin No. G1850. Lincoln Neb. USA.

Liu H, Qi Z, Zhang PZ, Song WT, Kou J, Zhang WJ, Yu, JL (2012). Response of photosynthesis and chlorophyll fluorescence to drought stress in two maize cultivars. African Journal of Agricultural Research 7:4751-4760.

Lu M, Xie CX, Li XH, Hao ZF, Li MS, Weng JF, Zhang DG, Bai L, Zhang SH (2011). Mapping of quantitative trait loci for kernel row number in maize across seven environments. Molecular Breeding 28:143-152.

Lyimo SD, Massawe FA, Owenya MZ, Mushi P, Sulumo P (2001). Report presented to the National Seed Release Committee meeting held in November 2000 at Selian Agricultural Research Institute, Arusha, Tanzania: Selian Agricultural Research Institute.

Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohrabi Y (2010). Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian Journal of Crop Science 4:580-585.

Magehema OA, Ladislaus CB, Mkoma SL (2014). Implication of rainfall variability on maize production in Morogoro, Tanzania. International Journal of Environmental Science 5:1077-1086.

Monneveux P, Sanchez C, Beck D, Edmeades GO (2006). Drought tolerance improvement in tropical maize source populations: evidence of progress. Crop Science 46:180-191.

SAS Institute Inc. (2009). The SAS system for windows, Version 9.2. SAS Institute Inc. Cary, NC, USA.

Wei L, Zhang D, Xiang F, Zhang Z (2009). Differentially expressed miRNAs potentially involved in the regulation of defense mechanism to drought stress in maize seedlings. International Journal of Plant Science 170:979-989.

Xu J, Yuan Y, Xu Y, Zhang,G, Guo X, Wu F, Wang Q, Rong T, Pan G, Cao M, Tang Q, Gao S, Liu Y, Wang J, Lan H, Lu Y (2014). Identification of candidate genes for drought tolerance by whole genome resequencing in maize. BMC Plant Biology 14:83.

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Full Length Research Paper

A clue for generating a new leucine-rich repeat gene in maize

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Plant leucine-rich repeat (LRR) proteins play an important role in cell adhesion and signaling, neuronal development, disease resistance response and pathogen recognition. Therefore, the origin and evolution of the LRR genes had been studied deeply. However, there were no evidence of generating new LRR genes. In this study, the genomic and amino acid sequences of the LRR genes or proteins were aligned based on Maize GDB and NCBI databases. The result showed that the part sequences of GRMZM5G851515 and part sequences of GRMZM2G167560 were consistent with parts of GRMZM2G343449. It was indicated that GRMZM2G343449 generated from GRMZM5G851515, GRMZM2G167560 and other genes. Evolution analysis also supported that GRMZM2G343449 and homologous genes were newborn. Meanwhile, they existed in only angiosperm that is closely related to human life, suggesting that they might be retained through the artificial selection. GRMZM2G343449 and its homologous genes in Poales were an independent branch of evolution which might be related with environment adaptability because there were more diseases and insect pests in the growth and development of crops than those of other species. The results indicated that GRMZM2G343449 and its homologous genes generated from stress resistance. This study provided key information for finding new generated LRR, which might be a clue for searching newborn genes from maize and other species in the big data era currently.

Key words: Leucine-rich repeat, evolution, generation, maize

INTRODUCTION

Protein containing leucine-rich repeat (LRR) domain (PLRR) existed in all life forms, from viruses to eukaryotes (Bella et al., 2008). Cell adhesion and signaling (Hohenester et al., 2006; Kresse and Schonherr, 2001), neuronal development (Chen et al., 2006), disease resistance response and pathogen recognition in plants (Gay and Gangloff, 2007; Martinon and Tschopp, 2005; West et al., 2006) are very important for the plant life. Currently, three typical PLRRs are plant receptors, FLS2 (Gomez-Gomez and Boller, 2000), EFR (Zipfel et al.,

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2006) and Xa21 (Song et al., 1995) which showed that PLRR is important for plant life, especially for disease resistance.

PLRR had different types based on the other different conversed domains (Bella et al., 2008). PLRRs contained tandems of two or more LRRs forming the continuously expanding LRR superfamily (Buchanan and Gay, 1996). For example, there were six PLRR types, such as LRR-Transmembrane (T)-Protein kinase (PK) (LRR-T-PK), LRR-PK, NB-LRR, LRR-T, LRR, and ATPases (A)-LRR (A-LRR) in maize (Li et al., 2016). More than 200 PLRR were distributed on the whole chromosomes of maize (Li et al., 2016). LRR domain was an essential part of PLRR. It was one of the six prolific types of protein which was a widespread structural motif (Andrade et al., 2001). The solvent-exposed amino acid residues of LRR in some PLRRs were involved in recognizing pathogens (Bergelson et al., 2001). Therefore, the study of evolutionary dynamics of PLRR is important for plant disease resistance.

In the evolution of LRR, The LRR existed in Eubacteria, Archaebacteria, Protista, Fungi, Plantae and Animalia (Yue et al., 2012). The number of LRR in Eubacteria, Archaebacteria and Fungi was far less than that in Plantae and Aminalia (Yue et al., 2012). It indicated that the evolution tendency of the LRR was similar to the tendency of evolving organisms and the evolution analysis of the LRR was important for the whole kingdom.

Previous studies showed that LRR and NBS domains existed before the split of prokaryotes and eukaryotes and the fusion of LRR and NBS domain was observed only in land plant lineages (Yue et al., 2012). However, there were no evidences to explain how to generate a new PLRR currently. In this study, we used three maize proteins for explaining the generation of a new PLRR gene, although the evidences were not integrated. Meanwhile, we analyzed the elevation of the new generated PLRR. This study aims to provide a clue for other research on generating new genes in the era of big data currently.

METHODOLOGY

Obtaining data

The genomic sequences and amino acid sequences were downloaded from the MaizeGDB (http://www.maizegdb.org/) (Lawrence et al., 2004) and NCBI database (http://www.ncbi.nlm.nih.gov/guide/) (Gene-duplication events of LRR genes (Li et al., 2016)).

Data analysis

SMART protein motif analysis (http://smart.embl-heidelberg.de/) (Letunic et al., 2012; Schultz et al., 1998) was used. The sequence alignment was carried out using DNAman Version 6.0. (Higgins and Sharp, 1989).

Data sampling

For each species, protein entries matching the LRR domain of GRMZM2G343449 in the NCBI database (http://www.ncbi.nlm.nih.gov/guide/) were identified as LRR-encoding protein using the blastp search with an *E*-value cut-off of 10^{-4} (Eddy, 1998; Yue et al., 2012). For all LRR-encoding proteins in our data set, the amino acid sequences were aligned and constructed as a phylogenetic trees using MEGA6 with the bootstrap of 1000 replicates (Tamura et al., 2013).

RESULTS

New born for one PLRR based on the DNA and protein sequences

On the basis of gene-duplication events of LRR genes (Li et al., 2016), we only found that GRMZM2G343449 on chromosome 4 and GRMZM5G851515 on chromosome 2, their amino acid sequences of N-terminals were identical among the duplication LRR genes. The identical region consisted of 295 amino acids (Figure 1). The percentages of identical continuous amino acids were 89.9 and 74.1% in GRMZM2G343449 and GRMZM5G851515, respectively. GRMZM2G343449 possessing 328 amino acids was on bin4.06, whereas GRMZM5G851515 possessing 398 amino acids was on bin 2.04 (Table 1). Both of them had four introns and belonged to the LRR-type (Figure 2A and 2B). Identical amino acids were not equal to identical DNA sequence. Therefore, the nucleic acid sequences of GRMZM2G343449 and GRMZM5G851515 were subsequently aligned. The result showed that there were 1298bp identical nucleic acids between GRMZM2G343449 and GRMZM5G851515 at the 3'-terminal except for six SNPs. Meanwhile, most different regions of the two proteins were in the first exon (Figure 3), suggesting that the first exon of GRMZM2G343449 or GRMZM5G851515 might derived from another encoded protein or or other unknown fragment on Chromosomes.

Looking for origins of generating one PLRR

To detect which gene was the origin, we blasted the DNA sequences of the two genes in NBCI and MaizeGDB databases, respectively. The references were the default values. On the basis of the about 13Gb genome from MaizeGDB that consist of four types of bases (A, T, C and G), more than 17 continuous DNAs might be a new gene using the power method of math. To a certain extent, continuous 170bp DNAs stand for a new gene in maize. There were no genes that had more than 170 bp identical DNAs to GRMZM5G851515. However, GRMZM2G167560 (NCBI accession number: EU972243) had 200 bp identical DNAs to 851th bp-1050th bp of GRMZM2G343449 (Figure 4), suggesting that GRMZM2G343449 might consist of GRMZM2G167560,

Figure 1. The amino acid sequence alignment of GRMZM2G343449 and GRMZM5G851515. The amino acids in Black background mean same amino acids between the two proteins.

Table 1. The details of GRMZM2G343449_T01 and GRMZM5G851515_T01 on chromosomes.

Maize DGB ID (Ensembl)	Chromosome	Bin	Number of amino acid
GRMZM2G343449_T01	Chr4:162859562..162862618	4.06	328
GRMZM5G851515_T01	Chr2:65851212..65855212	2.04	398

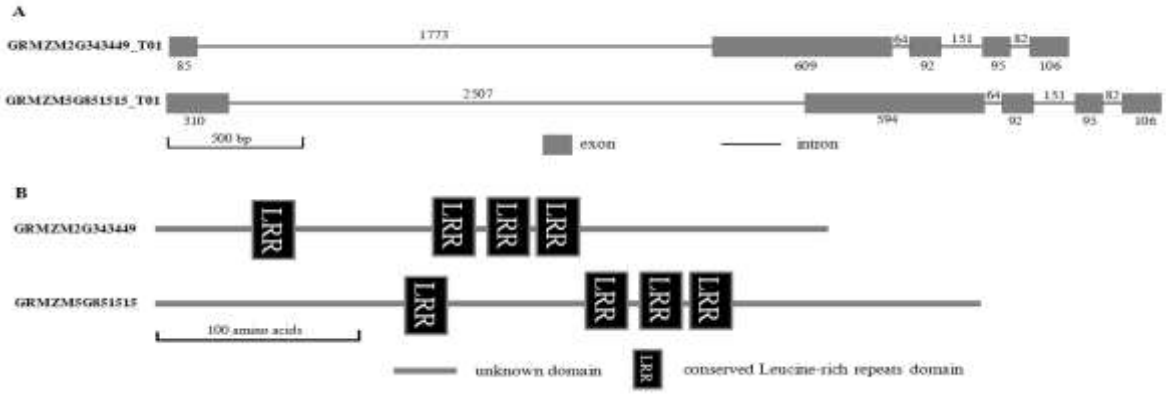

Figure 2. The structures of genes and proteins of GRMZM2G343449 and GRMZM5G851515. A, The structure of genes GRMZM2G343449 and GRMZM5G851515. B, The structure of proteins GRMZM2G343449 and GRMZM5G851515.

parts of GRMZM5G851515 and other DNA sequences with unknown origins (Figure 5). It is indicated that GRMZM2G167560 and GRMZM5G851515 existed before generating GRMZM2G343449. It was a clue for analyzing how new LRR or other genes were created in the period of big data.

The evolution analysis of the new generated PLRR

On the basis of an *E*-value cut-off of 10^{-4}, 23 homologous genes of GRMZM2G343449 were from 22 species in angiosperm except for GRMZM2G343449 in maize, whereas were not from gymnosperm, bryophyte, fungi and animals (Figure 6). It indicated the GRMZM2G343449 and its homologous were new-born and angiosperm-specific. The 23 species were closely related to human life. Among these species, most of them were edible. Though monocotyledon was not separated from dicotyledon completely based on clustering result, the species in Poales were clustered into together, which were distinguish from other orders (Figure 6). It indicated that the GRMZM2G343449 and its homologous genes in Poales were relatively independent in the process of

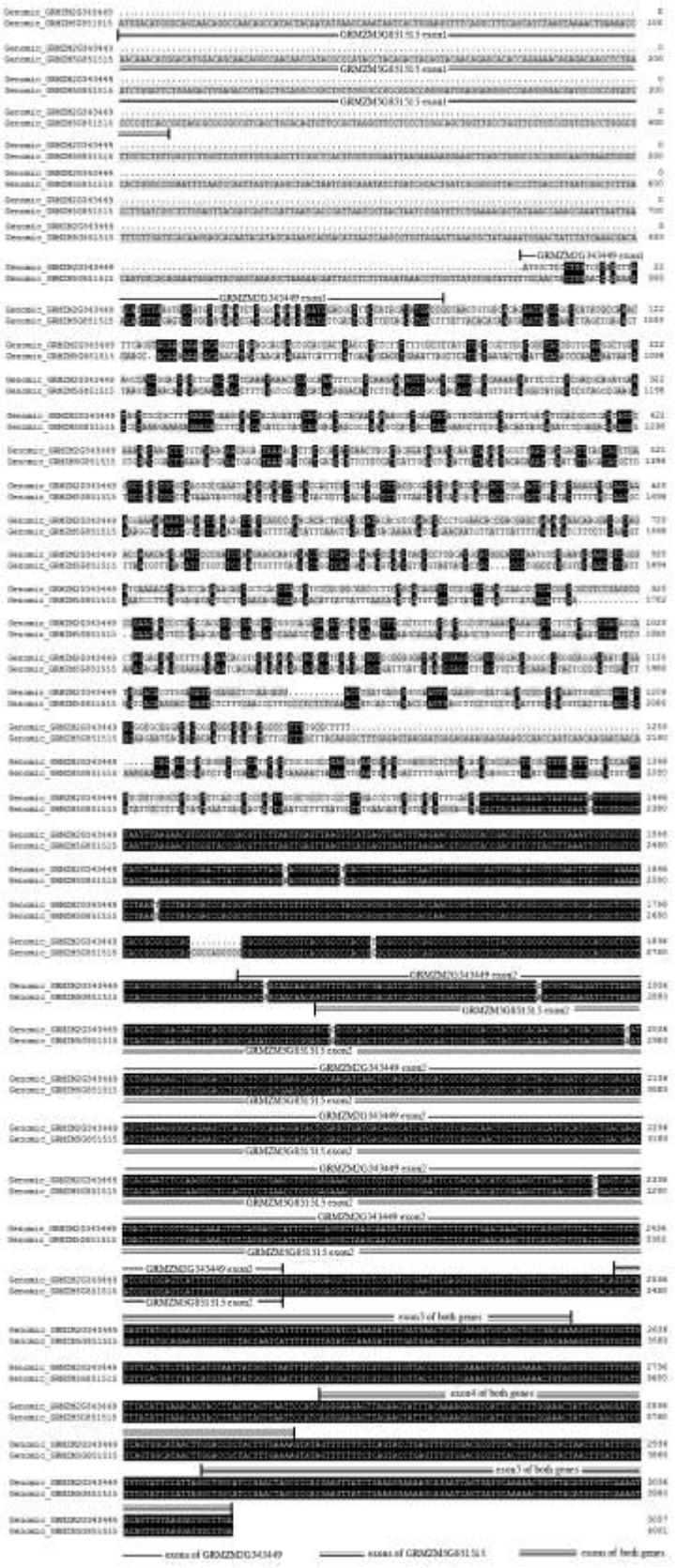

Figure 3. The genomic alignment of GRMZM2G343449 and GRMZM5G851515. Black bases mean same bases between the two genes.

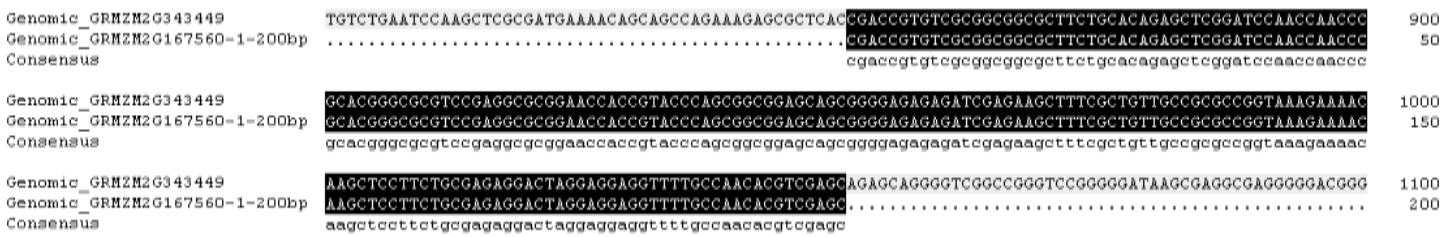

Figure 4. The genomic alignment of GRMZM2G343449 and GRMZM2G167560 at the 5' terminal. Black bases mean same bases between the two genes.

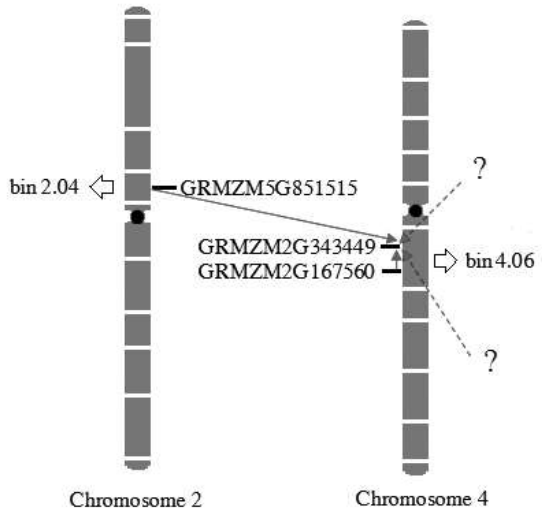

Figure 5. The mimetic diagram of generating GRMZM2G343449. ? Means unknown genes or fragments on chromosomes.

evolution.

DISCUSSION

LRR domain exists before the split of prokaryotes and eukaryotes (Yue et al., 2012). However, the LRR protein GRMZM2G343449 might be born in modern times which might be born after GRMZM2G167560, part of GRMZM5G851515. It might result from the help of transposons because transposons usually contributed into the generation of new genes. There was a large number of transposons in maize, such as Helitron transposons (Li and Dooner, 2009), Ac/Ds (Lazarow et al., 2013) and Mutator (Walbot et al., 1988). They might result into the generation of GRMZM2G343449.

In plant, LRR domains of several R-proteins is the major determinants of recognizing the specificity of Avr factors (Jones and Jones, 1997; Ellis et al., 2000; Leister and Katagiri, 2000). Amino acids in the LRR might also influence the interaction with host factors (Banerjee et al., 2001). Adaptive divergence among LRR proteins had been investigated in tomato (Parniske et al., 1997), rice (Wang et al., 1998) and Arabidopsis (Botella et al., 1998; McDowell et al., 1998; Noel et al., 1999). The LRR region often evolved at fast rates unusually (Bergelson et al., 2001). However, there were no direct evidences of evolution for LRR protein. In this study, the generation of GRMZM2G343449 provided the clue of evolution for LRR protein. It is a clue on how to born new LRR or other genes based on current big data.

Adaptive divergence and allelic polymorphism were the two types of evolutionary dynamics (Bergelson et al., 2001). The adaptive variants coexist with other alleles (Bergelson et al., 2001). R-proteins possessing LRR domains were usually associated with recognize pathogens (Bergelson et al., 2001). The generation of GRMZM2G343449 might result from the interaction of

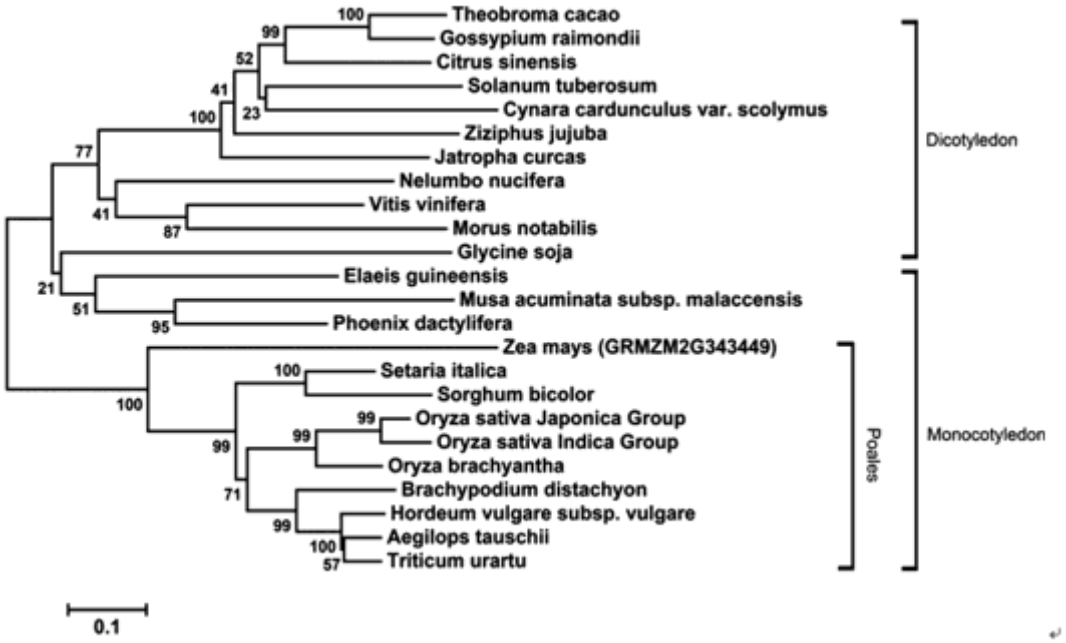

Figure 6. The phylogenetic tree of GRMZM2G343449 and its homologous genes. The NCBI ID of homologous gene from *Setaria italic* is XP_004982915.1, from *Sorghum bicolor* is KXG38364.1, from *Oryza brachyantha* is XP_006662430.2, from *Oryza sativa Japonica* Group is XP_015612820.1, from *Oryza sativa Indica* Group is EAY78879.1, from *Aegilops tauschii* is EMT14287.1, from *Hordeum vulgare* subsp. *Vulgare* is BAJ94896.1, from *Triticum urartu* is EMS46943.1, from *Brachypodium distachyon* is XP_003574034.1, from *Aegilops tauschii* is EMT33856.1, from *Musa acuminata* subsp. *malaccensis* is XP_009390803.1, from *Phoenix dactylifera* is XP_008801287.1, from *Theobroma cacao* is XP_007029633.1, from *Ziziphus jujube* is XP_015895303.1, from *Nelumbo nucifera* is XP_010249615.1, from *Solanum tuberosum* is XP_015163330.1, from *Setaria italic* is XP_004961982.1, from *Nelumbo nucifera* is XP_010241269.1, from *Phoenix dactylifera* is XP_008801288.1, from *Vitis vinifera* is XP_010646797.1, from *Morus notabilis* is XP_010106115.1, from *Glycine soja* is KHN31501.1, from *Jatropha curcas* is KDP35941.1, from *Elaeis guineensis* is XP_010910731.1, from *Gossypium raimondii* is XP_012470043.1, from *Cynara cardunculus* var. *scolymus* is KVI00715.1, from *Elaeis guineensis* is XP_010908122.1, and from *Citrus sinensis* is XP_015387143.1.

maize and pathogen. It indicated that GRMZM2G343449 might be associated with pathogen resistance of maize.

GRMZM2G343449 and its homologous genes existed in only 23 species of angiosperm, which does not exis extensively in angiosperm. These species were closely related to human life. It suggests that not only GRMZM2G343449 and its homologous genes might be new-born, but also they might be retained through the artificial selection. The homologous genes of GRMZM2G343449 in Poales were independent branch of evolution, it might be related with environment adaptability because there are more diseases and insect pests in the production of crops than in other species. It further indicated that GRMZM2G343449 and its homologous genes might be associated with stress resistance.

Conclusion

The current study revealed that GRMZM2G343449 might be generated from GRMZM5G851515, GRMZM2G167560 and other genes. Evolution analysis also supported that GRMZM2G343449 as an homologous genes might be newborn and retained through the artificial selection. GRMZM2G343449 and its homologous genes in Poales being an independent branch of evolution indicated that GRMZM2G343449 and its homologous genes generated from stress resistance. This study provided a clue for searching newborn genes from maize and other species

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Andrade A, Perez-Iratxeta C, Ponting P (2001). Protein repeats: Structures, functions, and evolution. Journal of Structural Biology 134:117-131.

Bella J, Hindle L, McEwan A, Lovell C (2008). The leucine-rich repeat structure. Cellular and Molecular Life Sciences 65:2307-2333.

Bergelson J, Kreitman M, Stahl E, Tian D (2001). Evolutionary dynamics of plant R-genes. Science 292:2281-2285.

Botella MA, Parker JE, Frost LN, Bittner-Eddy PD, Beynon JL, Daniels MJ, Holub EB, Jones JD (1998). Three genes of the Arabidopsis RPP1 complex resistance locus recognize distinct Peronospora parasitica avirulence determinants. Plant Cell 10:1847-1860.

Buchanan SG, Gay NJ (1996). Structural and functional diversity in the leucine-rich repeat family of proteins. Progress in Biophysics & Molecular Biology 65: 1-44.

Chen Y, Aulia S, Li L, Tang BL (2006). AMIGO and friends: an emerging family of brain-enriched, neuronal growth modulating, type I transmembrane proteins with leucine-rich repeats (LRR) and cell adhesion molecule motifs. Brain Research Reviews 51: 265-274.

Eddy SR (1998). Profile hidden Markov models. Bioinformatics 14: 755-763.

Ellis J, Dodds P, Pryor T (2000). Structure, function and evolution of plant disease resistance genes. Current Opinion in Plant Biology 3:278-284.

Gay NJ, Gangloff M (2007). Structure and function of Toll receptors and their ligands. Annual Review of Biochemistry 76:141-165.

Gomez-Gomez L, Boller T (2000). FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. Molecular Cell 5:1003-1011.

Higgins DG, Sharp PM (1989). Fast and Sensitive Multiple Sequence Alignments on a Microcomputer. Computer Applications in the Biosciences 5:151.

Hohenester E, Hussain S, Howitt JA (2006). Interaction of the guidance molecule Slit with cellular receptors. Biochemical Society Transactions 34:418-421.

Jones DA, Jones JDG (1997). The Role of Leucine-Rich Repeat Proteins in Plant Defences. Advances in Botanical Research 24(8):89-167.

Kresse H, Schonherr E (2001). Proteoglycans of the extracellular matrix and growth control. Journal of Cellular Physiology 189: 266-274.

Lawrence CJ, Dong Q, Polacco ML, Seigfried TE, Brendel V (2004). MaizeGDB, the community database for maize genetics and genomics. Nucleic Acids Research 32:D393-397.

Lazarow K, Doll ML, Kunze R (2013). Molecular biology of maize Ac/Ds Elements: An Overview. Methods in Molecular Biology 1057:59-82.

Leister RT, Katagiri F (2000). A resistance gene product of the nucleotide binding site -leucine rich repeats class can form a complex with bacterial avirulence proteins in vivo. Plant Journal 22: 345-354.

Letunic I, Doerks T, Bork P (2012). SMART 7: recent updates to the protein domain annotation resource. Nucleic Acids Research 40:D302-D305.

Li WT, Xiang K, Zhang ZM, Yuan GS, Lin HJ, Pan GT (2016). Systematic analysis of leucine-rich repeat disease resistance genes in maize. Maydica 61(1):10.

Li Y, Dooner HK (2009). Excision of Helitron transposons in maize. Genetics 182(1):399-402.

Martinon F, Tschopp J (2005). NLRs join TLRs as innate sensors of pathogens. Trends in Immunology 26:447-454.

McDowell JM, Dhandaydham M, Long TA, Aarts MG, Goff S, Holub EB, Dangl JL (1998). Intragenic recombination and diversifying selection contribute to the evolution of downy mildew resistance at the RPP8 locus of Arabidopsis. Plant Cell 10:1861-1874.

Noel L, Moores TL, van Der Biezen EA, Parniske M, Daniels MJ, Parker JE, Jones JD (1999). Pronounced intraspecific haplotype divergence at the RPP5 complex disease resistance locus of Arabidopsis. Plant Cell 11:2099-2112.

Parniske M, Hammond-Kosack KE, Golstein C, Thomas CM, Jones DA, Harrison K, Wulff BB, Jones JD (1997). Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the Cf-4/9 locus of tomato. Cell 91:821-832.

Schultz J, Milpetz F, Bork P, Ponting CP (1998). SMART, a simple modular architecture research tool: identification of signaling domains. Proceedings of the National Academy of Sciences of the United States of America 95:5857-5864.

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P (1995). A receptor kinase-like protein encoded by the rice disease resistance gene, Xa21. Science 270:1804-1806.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30:2725-2729.

Walbot V, Britt AB, Luehrsen K, McLaughlin M, Warren C (1988). Regulation of mutator activities in maize. Basic Life Sciences 47:121-135.

Wang GL, Ruan DL, Song WY, Sideris S, Chen L, Pi LY, Zhang S, Zhang Z, Fauquet C, Gaut BS, Whalen MC, Ronald PC (1998). Xa21D encodes a receptor-like molecule with a leucine-rich repeat domain that determines race-specific recognition and is subject to adaptive evolution. Plant Cell 10:765-779.

West AP, Koblansky AA, Ghosh S (2006). Recognition and signaling by toll-like receptors. Annual Review of Cell and Developmental Biology 22:409-437.

Yue JX, Meyers BC, Chen JQ, Tian D, Yang S (2012). Tracing the origin and evolutionary history of plant nucleotide-binding site-leucine-rich repeat (NBS-LRR) genes. New Phytologist 193:1049-1063.

Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JD, Boller T, Felix G (2006). Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. Cell 125:749-760.

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Full Length Research Paper

Evaluation of different methods to control invasive alien grass weeds in a degraded area

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The objective of this study was to evaluate the efficiency of different methods of exotic grass control in a degraded area in the urban area of Diamantina, state of Minas Gerais, Brazil. The experiment was carried out in a randomized block design, with the following methods of control of invasive grasses: T1) manual; T2) mechanical; T3) chemical; and T4) chemical + mechanical. After one hundred days, regenerants of alien invasive species biomass were sampled for the quantification of fresh biomass and dry biomass. The results found fresh biomass vary among the control methods, with higher values from mechanical control, followed by the combination of mechanical + chemical control. Chemical and manual methods had the lowest fresh biomass production, indicating that they were more effective in controlling the invasive grasses. The production of dry matter did not differ significantly among the methods of exotic grass control, on Tukey test, at 5% significance.

Key words: *Urochloa decumbes*, *Melinis minutiflora*, biomass, restauration.

INTRODUCTION

Brazil is one of the most biodiverse countries on the planet (Forzza et al., 2012). However, with the introduction of alien invasive weeds and the lack of effective prevention and control policies, biological invasion has become the major agent of global change (Early et al., 2016). The establishment, adaptation and dispersion of these species cause great changes in the functioning of natural ecosystems due to their aggressiveness and high invasiveness (D'Antonio and Vitousek, 1992; Rodovalho and Nardoto, 2014; Martins et al., 2017) as well as cause changes in abundance, population size, composition genetics, and community structure (Byers et al., 2002).

The invasive species are present in Brazilian soils since the colonial period, being verified in much of the Brazilian territory, their dispersion threatens to biodiversity, being the Cerrado one of the most affected Biomes (Xavier et al., 2017).

Species of African origin as *Urochloa decumbes* (Stapf) RD, Wabster, *Brachiaria decumbens* Staf. and *Melinis minutifliora* P. Beauv., were introduced for commercial purposes and/or by accident and began to occupy large tracts displacing native species due to their aggressiveness and competitive power. Such invasions

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have contributed to the de-characterization of natural areas and changes to the original vegetation composition (Weber, 2017).

In this sense, the control of these species is of extreme importance. Efforts have involved the implementation of soil and cultural management practices and/or preventive mechanical, biological and chemical means for decreasing the competitive advantage of invasive species with the purpose of the resurgence and reestablishment of local endemic and native species (Vivian et al., 2008). Consideration of cost analysis and control strategies is necessary to support decision-making by ensuring natural ecosystem management when benefits exceed the costs (King et al., 1998; Born et al., 2005).

This paper aims to evaluate the effectiveness of different types of grass control (manual, mechanical and chemical) in a degraded area.

METHODOLOGY

The study was performed from July 2015 to March 2016 in an area of approximately 1.0 ha located a 18° 12' 18.85"S, 43° 34' 9.12" W, on Universidade Federal dos Vales do Jequitinhonha and Mucuri-Campus JK, in the Espinhaço Mountain Range, in the southeast portion of the municipality of Diamantina, Minas Gerais, Brazil.

The average altitude is 1,296 m and the climate is mesothermic, with rainy summers and dry winters. The mean annual temperature varies between 17.4 and 19.8°C (INMET, 2016) and the annual precipitation is around 1,400 mm and occurs among the rainy season (November to January), the dry season (May to September) and the months of transition. Averages for temperature during the experimental period were 24°C in August and September and 27°C in October and November. Average for precipitation of 241 mm was recorded, with 81 mm in August, September and October, and 160 mm in November 2015 based on data from the Instituto Nacional de Meteorologia (INMET, 2016). The predominant soil class of the study site is Entisols, whose main characteristics are sandy texture with simple grain structure, which gives it high macro-porosity, resulting in low saturation capacity and low retention of water available for plants.

The typical vegetal covers of this pedoenvironment are rupestrian cerrado and field formations, ecotypes adapted to seasonal water deficit, since most of the species have a root system adapted to absorb water at great depths (Machado et al., 2013).

The site functioned for solid waste disposal in the urban area of Diamantina, Minas Gerais, and finished in 2002. Currently, it is in the process of recovery. After decommissioning as a controlled landfill, there were concerns about re-vegetating and erosion control due to the exposure of the substrate. Initially random seedlings were introduced in an attempt to form vegetation nuclei besides exotic grass species: *U. decumbes* (Stapf) RD, Wabster and *M. minutiflora* P. Beauv., with the intention of promoting rapid substrate coverage (Machado et al., 2013). At present, the experimental area have predominance of these exotic grasses besides, in their vicinity, some vegetation nuclei with the presence of ruderal tree species (*Psidium guajava* L., *Ricinus communis* L. and *Vernonia polysphaera* Baker), and some native species *Eremanthus incanus* (Less.) Less, *Dalbergia miscolobium* Benth, *Stryphnodendron adstringens* (Mart.) Coville and *Tabebuia ochracea* (Cham.) Standl.

Due to these features, intervention by means of control was necessary since these grasses prevented the development of the seed bank and were in direct competition with the tree species established there, making it difficult to interconnect fragments, thus hampering the recovery processes.

The experiment was performed in a randomized block design, four blocks with dimensions of 16 m × 40 m (640 m^2) as shown in Figure 1. The treatments were constituted by the following methods of invasive grass control: T1) manual; T2) mechanical; T3) chemical; and T4) chemical + mechanical. All of the blocks had a predominance of individuals of *U. decumbes* and *M. minutiflora*. Each block had distinct characteristics, but was internally homogeneous. In each one, 16 plots with dimensions of 4m × 10 m (40 m^2) were allocated, which were randomly treated by the different methods, with four replications each, in an attempt to control the grasses.

The chemical method used pressurized CO_2 and a 20l model Jacto-PJH sprayer with capacity for glyphosate herbicide application. *Roundup Original*®, containing 36% glyphosate, was applied using the recommended dosage of 4.0 l/ha with a volume of solution of approximately 7.5 L per block (120 l/ha). The herbicide was applied to the locally established grasses that were about 1 m tall in August 2015. The mechanical method used a KAWASHIMA TEKNA AL330TH gasoline powered motorized trimmer. The manual method used a hoe.

The amount of time spent for each plot treatment was recorded and averages calculated for each control method. The labour required for the application of the treatments involved two people for the manual and mechanical methods and a technician for the herbicide application of the chemical method. The elimination of individuals of *U. decumbes* and *M. minutiflora* began in August 2015. For manual method was performed, all individuals of the mentioned species were removed, leaving the substrate totally exposed. In the experimental units where the mechanical and/or chemical methods were adopted, the eliminated individuals were maintained *in loco* in order not to expose the substrate.

The regenerate grasses were sampled one hundred days after the application of the control method treatments. In each experimental unit, 12 points were randomly distributed for the collection of regenerated grasses with the aid of 1.0m-square iron frame, totaling 48 units per treatment. The grasses were cut close to the substrate with scissors and other species were kept in place. After collection, the fresh material was packed in paper bags, weighed and then subjected to a forced-air circulation oven at 65° C for 72 hours to obtain the dry weight.

The proposed transformation by Box and Cox (1964), was applied only to dry biomass data, expressed in kg/ha, in order to meet the normality assumptions according to the Shapiro-Wilk homogeneity of variances by Bartlett, independence of waste by Durbin-Watson and the additivity by Tukey. The value of the lambda transform parameter (λ) of Box-Cox was 0.48.

The fresh biomass and dry biomass data were submitted for analysis of variance (F test) and the means were compared by the Tukey test, both at 5% significance. All statistical analyses were compiled and compared with the aid of R © Version.3.2.3.

RESULTS

The time spent executing the different control methods varied. The manual method had a mean time of 120 min, followed by the mechanical and chemical methods, with mean times of 4 and 2 min, respectively. After the execution of the different control methods (manual, chemical and mechanical), plants were allowed to emerge freely, without the interference of any cultural practice, addition of fertilizer or irrigation, in order not to influence their development and survival.

Figure 1. Location of Diamantina in the state of Minas Gerais and Satellite Image Digital Globe (2015) of the part of the JK Campus of the Federal University of Vales do Jequitinhonha and Mucuri where the 4 experimental blocks were located. The colours of the circles define the type of method performed in each plot: yellow (chemical + mechanical methods); red (mechanical method); blue (manual method) and white (chemical method).

Table 1. Analysis of variance summary for fresh biomass and dry biomass (transformed data) using F-test statistic.

S.V.	D.F	M.S.	
		Fresh biomass	Dry biomass
Blocks	3	929670.0	854.0
Treatments	3	2528982.0*	845.7*
Residue	9	338975.0	468.2
CV_{exp} (%)	-	24.40	15.50

SV: Source of variant; D.F: degrees of freedom; M.S: middle square; *significant at 5% probability by the test F. CV_{exp}: Coefficient of experimental variation

The manual method exposed the substrate, which favours the development of seed banks and seedlings. During the entire experiment, 241 mm of precipitation was recorded, of which 160 mm was in November 2015. Due to the low precipitation in the period between September and October, no seedling development was observed. With the increase of precipitation in November, the seedlings could not compete with the grasses and eventually died.

In the plots where the mechanical and chemical control methods were implemented, the resulting material was kept in the areas to cover the substrate, thereby avoiding its exposure, which could contribute to the degradation of the already fragile area. The presence of this material does not permit sunlight from reaching the surface, which prevented the development of seedlings from the seed bank, as had occurred in the plots of the manual method.

The assumptions of normality, homogeneity of variances, residue independence and additivity were met. The coefficients of variation were less than 25%, relatively low for field experiments. Analysis of variance with the F test indicated a statistically significant difference between treatments ($p \leq 0.05$) both for the production of fresh biomass and that of dry biomass. Methods for the control of invasive grasses influenced biomass production 100 days after experimental installation (Table 1).

Figure 2 summarizes the data evaluated for (i) the production of fresh biomass (fb), as measured by initial weight after harvesting at 100 days and (ii) dry biomass (db), as measured by the weighing of post-dried material, obtained for the different control methods. The

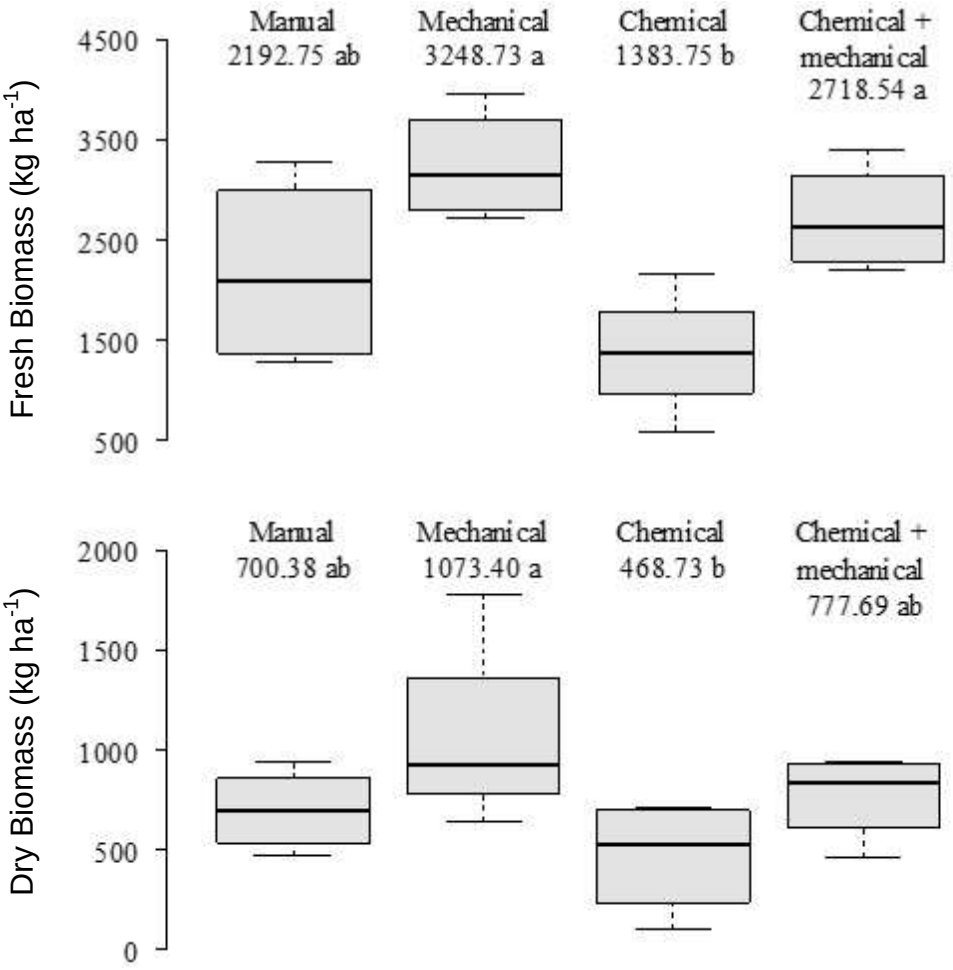

Figure 2. Quantification of fresh and dry biomass of *Urochloa decumbes* and *Melinis minutiflora* with different types of invasive control methods after 100 days. Means followed by the same letter do not differ by the Tukey test at 5% significance.

comparison of the means by the Tukey test showed a lower production of fresh biomass of invasive grasses when the manual and chemical control methods were used. These two methods and the combination of chemical and mechanical control promoted lower production of dry biomass of grasses (Figure 2).

DISCUSSION

The seed banks in the area are composed mostly of invasive herbaceous species and no shrub-tree species (Machado et al., 2013). Therefore, the increase in precipitation in November produced suitable conditions for the development of the seed bank, with a predominance of exotic grasses, thereby hindering the process of ecological succession of the study area.

The chemical method had lower biomass production and greater control of exotic grasses after 100 days, whereas the mechanical method had the opposite extreme. Thus, it is clear that the chemical method is the most effective at controlling invasive grasses since it produced less biomass over the period of 100 days than did the other types of methods. In addition, the chemical method required less manpower and time for its implementation.

Each herbicide has its particularity, the mode of action, either in the plants or the applied environment, being glyphosate (systemic) one of the most used herbicides, due to its versatility and effective control (Silva et al., 2013; Timossi et al., 2016). Generally, after the effect of the herbicide, the dead and dried plant materials provide soil cover and thus conditions conducive to the germination of plants in the seed bank. The use of action herbicides other than glyphosate, such as paraquat (contact), which only acts via contact with plants, can compromise the control of invasive grasses.

Several works have had success using the chemical

method and they are widely used and recommended for large open areas, which generally have greater potential for re-infestation of grass (Mantoani et al., 2016). According to them, the cost of chemical control is 34.1% lower than mechanical treatment. The chemical control employed in the present study, when done successively, provides a reduction in infestation and a consequent decrease of cost due to the smaller amount of herbicide used. This is why reforestation companies do prefer chemical treatments.

On the other hand, chemical control can become costly and requires caution since the use of herbicides in the same area over continuous periods may favour the establishment of resistant weed species (Heap*,* 2014). In areas close to fragments of vegetation, cover crops are better than herbicide application. The planting of species of fast initial growth and high production of biomass will serve to shade the area and consequently the weeds, thus leading to their death (Gomes and Christoffoleti, 2008).

The other types of control methods used in the present study (mechanical and manual) allow close cutting or complete removal of the vegetative material, which can lead to intense exposure of the substrate and lead to intensification of erosion. An alternative would be the use of manual control in small areas under the early stages of invasive species development. The results of a study showed that the control of weeds in the period of 30 and 40 days after emergence provided a reduction of 45.1 and 84.0% of dry matter accumulation of the invasive species as compared to a control area lacking a control method (Galon et al., 2008; Noce et al., 2010). Such action reduces the amount of biomass and labour costs.

The mechanical and mechanical + chemical methods both generated an accumulation of dead vegetation in the plots. This material can influence, in an antagonistic way, the development of the seed bank, and lead to increased humidity and decreased surface temperature. However, on the other hand, this material can function as a physical barrier, mechanical damages, erosion and release of substances with allelopathic properties. These factors prevent the development of other species as well as obstruction of passage from light to the soil/substrate (Costa et al., 2018). The presence of this dead vegetation may have other negative consequences for deactivated controlled landfill areas, such as the area of the present study, such as risk of fire due to the significant concentrations of methane gas released into the environment due to the intense decomposition of the various types of solid wastes.

These observations can contribute to taking decisions on the control of invasive species, specifically, the use of the mechanical method must coincide with the flowering of the weed species because it is the period that the weeds reserves are converted to the production of seeds, and thus possess limits resources for regrowth. Therefore, it is necessary to know the period when the target species is at its apex of growth, thus reducing its infestation and, in some cases, reducing or dispensing with the need of employing cultural practices. With the control of invasive species, it is necessary simultaneously introduce native species with the potential for revegetation, which is a gradual process of re-establishing local biodiversity, including genetic diversity. A study in mining areas degraded (about 1 km from the study area) indicates that the remaining vegetation is still having low floristic richness when compared with other studies carried out in the region (Pereira et al., 2015). The authors found species such as *Eremanthus erythropappus, E. incanus, Tibouchina candolleana* and *Tremble Yacf*. *parviflora* that even exist in the area of this study. They will contribute to the shading of the area, avoiding the accentuated proliferation of exotic grasses.

Therefore, in the study area continuous evaluation and the use of measures to control invasive grasses are necessary in order to minimize direct competition with native species. Furthermore, because it is a small area, the manual method is the best option due to the complete removal of the material, despite requiring a greater effort and time. The insertion of pioneer species could be beneficial by controlling invasive grasses in the medium term.

In the search for the most efficient methods of invasive grass control, further detailed studies of other methods of control are indispensable. Several factors can influence the response of these invasive species to control, especially in areas of solid waste deposits that present a huge variation due to the different deposited materials and stages of decomposition, which interfere directly with the properties of the substrate. This fact may affect directly the resilience of the environment, making the site unsuitable for development of any species and making it difficult to recompose the vegetation in these places.

Conclusion

Among the distinct types of methods used to control exotic grasses, the most practical and effective for the degraded area analysed in this study was the chemical method, resulting in less regenerated biomass. On the other hand, the mechanical method was the least effective, as evidenced by the greater production of biomass.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Born W, Rauschmayer F, Bräuer I (2005). Economic evaluation of biological invasions—a survey. Ecological Economics 55(3): 321-336.

Box GE, Cox DR (1964). An analysis of transformations. Journal of the Royal Statistical Society. Series B (Methodological) pp. 211-252.
Byers JE, Reichard S, Randall JM, Parker IM, Smith CS, Lonsdale WM, Hayes D (2002). Directing research to reduce the impacts of nonindigenous species. Conservation Biology 16(3):630-640.
Costa N, Rodrigues-Costa A, Coelho E, Ferreira S, Barbosa J (2018). Métodos de controle de plantas daninhas em sistemas orgânicos: breve revisão. Revista Brasileira de Herbicidas 17(1):25-44.
D'Antonio CM, Vitousek PM (1992). Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics 23(1):63-87.
Early R, Bradley BA, Dukes JS, Lawler JJ, Olden JD, Blumenthal DM, Sorte CJ (2016). Global threats from invasive alien species in the twenty-first century and national response capacities. Nature Communications 7:12485.
Forzza RC, Baumgratz JF, Bicudo CE, Canhos DA, Carvalho Jr AA, Coelho MA, Costa AF, Costa DP, Hopkins MG, Leitman PM, Lohmann LG (2012). New Brazilian floristic list highlights conservation challenges. BioScience 62(1):39-45.
Galon L, Pinto JJO, Rocha AA, Concenço G, Silva AF, Aspiazú I, Pinho CF (2008). Interference periods of Brachiaria plantaginea in corn crops in Southern Rio Grande do Sul. Planta Daninha 26(4):779-788.
Gomes Jr FG, Christoffoleti PJ (2008). Biologia e manejo de plantas daninhas em áreas de plantio direto. Planta Daninha 26(4):789-798.
Heap I (2014). Global perspective of herbicide-resistant weeds. Pest Management Science 70(9):1306-1315.
INMET (2016). Instituto Nacional de Meteorologia. Ministério da Agricultura, Brasil. http://www.inmet.gov.br/portal/index.php?r=estacoes/mapaEstacoes
King RP, Swinton SM, Lybecker, DW, Oriade CA (1998). The economics of weed control and the value of weed management information. In: Hatfield JL, Buhler DD, Stewart BA. (eds). Integrated Weed and Soil Management pp. 25-41.
Machado VM, Santos JB, Pereira IM, Lara RO, Cabral CM, Amaral CS (2013). Evaluation of the seed bank in a campestre cerrado area under recovery. Planta Daninha 31(2):303-312.
Mantoani MC, Dias J, Torezan JM (2016). Roçagem e aplicação de herbicida para controle de Megathyrsus maximus: Danos sobre a vegetação preexistente em um reflorestamento de 20 anos. Ciência Florestal 26(3):839-851.
Martins CR, Hay JDV, Scaléa M, Malaquias JV (2017). Management techniques for the control of *Melinis minutiflora* P. Beauv. (molasses grass): ten years of research on an invasive grass species in the Brazilian Cerrado. Acta Botanica Brasilica 31(4):546-554.
Noce MA, De Souza IF, Karam D, França AC, MacieL GM (2010). Influência da palhada de gramíneas forrageiras sobre o desenvolvimento da planta de milho e das plantas daninhas. Revista Brasileira de Milho e Sorgo 7:03.
Pereira IM, Gonzaga APD, Machado ELM, Oliveira MLR, Marques IC (2015). Colonizer vegetation structure in gravel mining degraded environment in Diamantina, MG, Brazil. Pesquisa Florestal Brasileira 35(82):77-88.
Rodovalho NL, Nardoto GB (2014). Distribuição dos trabalhos sobre capim-gordura no território brasileiro: uma analise histórico-espacial. Espaço e Geografia 17(1):97-113.
Silva UR, Timossi PC, Almeida DP, Lima SF (2013). Eficácia do glyphosate na dessecação de espécies de Urochloa. Revista Brasileira de Herbicidas 12(2):202-209.
Timossi PC, Almeida DP, Ramos AR, Carvalho Felisberto PA, Lima SF, Silva UR (2016). Glyphosate effectiveness in the burndown of signalgrass at two levels of biomass. Revista Brasileira de Herbicidas 15(4):313-322.
Vivian R, Fagan EB, Silva AA, Labonia V, Gimenes Jr M, Ruiz ST (2008). Dormência em sementes de plantas daninhas como mecanismo de sobrevivência – breve revisão. Planta Daninha 26(3):695-706.
Weber E (2017). Invasive plant species of the world: a reference guide to environmental weeds. Oxfordshire, UK. Boston, MA. CABI. 596p.
Xavier R, Leite MB, Silva-Matos DM (2017). Stress responses of native and exotic grasses in a Neotropical savanna predict impacts of global change on invasion spread. Austral Ecology 42:562-576.

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Full Length Research Paper

Analysis of energy input-output of irrigated rice production in Jere Bowl Borno State, Nigeria

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The study analyzed energy input-output of irrigated rice production at the Jere Bowl Borno State, Nigeria. One hundred and thirty farms were sampled through multistage sampling procedure. The data collected were analyzed using descriptive statistics and energy equations. The result showed that total of 36,397.85 MJ/ha of energy was consumed in the irrigated rice production with the highest energy input taken up by irrigation water (48.51%) and fuel (23.58%). The forms of energy estimated showed that direct energy contributed much to rice production (76.21%) than the indirect energy with 23.77%; similarly, renewable energy constituted higher energy (59.98%) to the production system, while non-renewable energy constituted only 39.99% of energy to the production system. The results on energy indices of rice production indicated energy ratio of 2.47, specific energy of 31.38 MJ/kg, energy productivity of 0.147 kg/MJ, water productivity of 0.103 kg/M^3 and net energy of 53,598.71 Mj/ha. The lower values of energy indices indicate inefficient use of energy. The study recommended that farmers should adopt efficient and cost effective irrigation system which will ensure efficient utilization of energy.

Key words: Input-output, energy, direct energy, indirect energy, renewable energy, indices, water productivity.

INTRODUCTION

Rice is a very important food crop in the world because it is the second largest cereal consumed after wheat which provides staple food for more than half of the world's population with about 80% of its food calorie requirements (Inuwa et al., 2011). Rice is cultivated and consumed in all parts of Nigeria. Rice production produces and consumes energy in form of bio-energy. The resource inputs used in rice production are composed of energy. This energy is called bio-energy or energy input. The ability of the input to function in the production of rice is as result of the energy input. The production of rice in a system with high yield targets cannot be achieved without energy inputs such as energy in seed, fertilizer, pesticides and labour to the system. This energy is further categorized into direct, indirect, renewable and non-renewable energy. Direct energy is the energy consumed directly in the rice production e.g. human labour, animal labour, fossil fuels, and electricity. The sources of these energy are human, animal, petrol, diesel and water required to perform different tasks in the

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crop production processes such as field preparation, cultural practices, irrigation, harvesting, threshing and transportation. However, the energy that is used in manufacturing, packaging and transportation of different farm inputs such as seed, fertilizers, farmyard manure, pesticides and other chemicals and machineries are called indirect energy. The energy that come from human in form of labor, from animal in form of power, from seed and farmyard manure are classified as renewable energy, however, the non-renewable energy sources are petrol, diesel, electricity, chemicals and machinery. On the other hand, the petrol, diesel and electricity falls under the category of commercial energy but the human, animal and farmyard manure fall under the category of non-commercial energy (Singh et al., 2007). Effective planning for the use of the energy in the process of rice production is imperative to attain efficient performance of rice with less environmental pollution. The extent to which irrigated rice production in Jere Bowl Borno State consumed energy is unknown. This study seeks to unravel this information.

Objectives of the study

The objectives of the study were to:

(1) examine the level of energy consumption in rice production in the study area;
(2) identify the forms of energy used in rice production in the study area; and
(3) estimate the energy indices of rice production in the study area.

METHODOLOGY

Study area and data collection

The study was conducted in Jere Bowl Borno State, Nigeria. It lies between latitudes 11° 40' and 12° 05'N and longitudes 13° 05' and 12° 20' E with a projected population of 277, 913 persons in 2017 based on 2.8% population growth rate (National Population Census, 2006). Jere Bowl has a cultivable land area of about 22,000 ha, out of which a gross area of 15,850 ha was identified as suitable for irrigated agriculture (Jibirin, 2010). The climate of the area is dry and hot for most part of the year with minimum temperature ranging from 15 to 20°C and maximum range of 37 to 45°C. The annual rainfall ranges from 500 to 700 mm characterized by high variability and intensity. The rainy season usually last from May to September with a relatively low humidity (Nigerian Metrological Agency (NMA), 2008). This is followed by a long dry season. The major river in the area is the Ngadda River which flows through Alau Dam where overbank flows occur. This resulted in the formation of the Jere Bowl (Nyanganji, 1994), which is generally referred to as Fadamas in Hausa language, meaning lowland, floodplain, and valley-bottom around a river.

Both primary and secondary data was used for this study. The primary data were collected with the aid of well-structured questionnaire which was administered to the respondents. The questionnaire contains information on the rice farm inputs (such as seed, fertilizer, labour, water, pesticides and fuel) and farm output (rice grain and straw). A total of 130 farmers were selected from four purposively selected communities using multi-stage sampling procedure. The sampling frame was rice farmers associations of Jere Bowl Borno State. The sample equation was used to determine the number of respondents from each of the community and was expressed as follows:

$$n = \frac{N}{N+1(\alpha)2} \quad (1)$$

where n = Sample size, N = Population, and α = Confidence interval.

Analytical techniques

The energy equivalents in Table 1 were used with Equations 1, 2 and 3 to convert the physical amount of inputs and outputs to energy forms expressed in Mj/ha.

Following Gevao et al. (2007), Karale et al. (2008) and Ann (2011), the equations are given as.

(1) Labour energy: The energy of labour in the production was estimated using the following equation:

$$\text{Lab.} = \frac{lb.T.Lf}{a} \quad (2)$$

where Lab = Energy of labour (mj/ha), Lb = Number of working labourers (No), T = Operating time (h), Lf = equivalent energy of labour (mj/h), and a = Area covered (ha).

(2) Energy of water**:** The energy of water used during irrigation in rice production, was determined using the following equation:

$$EW = \frac{DC.T.F.Eqf}{a} \quad (3)$$

where EW = Energy of water (MJ/ha), DC = Discharge capacity of the water pump (m^3/min), T = Time of water application (Min/application), F = Frequency of application (Number of time), Eqf = Energy Equivalent for water (MJ/M^3), and a = Area applied (ha).

(3) Energy per unit area for other production inputs such as fertilizer, fuel, pesticides and seed as well as the energy output was expressed as:

$$EID = RATE \cdot MATENF \quad (4)$$

where EID = Energy inputs (Mj/ha), RATE = Application rate of Input (unit/ha), and MATENF = Energy equivalent of input (Mj/unit).

Energy indices

To estimate the energy indices in rice production (such as energy ratio, energy productivity, specific energy and net energy), Equations 4, 5, 6 and 7 were used to satisfy objective iii of the study. Following Mohammadi et al. (2008) the energy indices was determined using the following equation:

$$\text{Energy ratio} = \text{Energy output (MJ/ha)} / \text{Energy input (MJ/ha)} \quad (5)$$

$$\text{Energy productivity} = \text{Rice output (kg/ha)} / \text{Energy input (MJ/ha)} \quad (6)$$

$$\text{Specific Energy} = \text{Total Energy output (MJ/ha)} / \text{Grain yield (kg/ha)} \quad (7)$$

Table 1. Equivalent energy conversion factors.

Energy use	Energy coefficient (MJ/unit)	Sources
Human Labour (h)	1.96 MJ/h	Gundogmus (2006)
Seed	17.5 MJ/kg	Singh et al. (2007)
Fertilizer (kg)		
Nitrogen	60.60 MJ/kg	Gundogmus (2006)
Phosphorus	11.10 MJ/kg	Gundogmus (2006)
Potassium	6.70 MJ/kg	Gundogmus (2006)
Pesticide (kg)		
Insecticide	199 MJ/kg	Gundogmus (2006)
Fungicide	92 MJ/kg	Gundogmus (2006)
Herbicide	238 MJ/kg	Gundogmus (2006)
Paddy (kg)		
Grain	14.57 MJ/kg	Iqbal (2007)
Straw	12.50 MJ/ kg	Iqbal (2007)
Water (M^3)	0.63 MJ/M^3	Gundogmus (2006)
Fuel (L)		
Petrol	37 MJ/L	Kaltsas et al. (2007)
Diesel	56.31 MJ/kg	Gundogmus (2006), Cherati et al. (2011) and Erdal et al. (2007)

Water Productivity = Grain Yield (Kg/ha) / Amount of Water Applied (M^3/ha) (8)

Net Energy = Energy output (MJ/ha) - Energy input (MJ/ha) (9)

It is assumed that if the value of energy ratio >1, it means production system is gaining energy, <1; it means production system is losing energy. Similarly, if the energy/water productivity ≥1, it means higher productivity and if <1 it means lower productivity (Falaye, 2013; Ibrahim and Ibrahim, 2012).

RESULTS AND DISCUSSION

Level of energy consumption in rice production

Amounts of inputs used and output produced in rice production for each item as well as their energy components per hectare are shown in Table 2. The result revealed that 766.59 h of human labour, 153 kg of seed, 82.07 kg of nitrogen, 15.18 kg of phosphorus, 15.18 kg of potassium, 2.71 L of pesticide, 29,817.76 m^3 of irrigation water and 232.09 L of fuel, per hectare were used for the production of rice in the study area. These are equivalent to, 1502.51, 2677.5, 4973.40, 168.51, 101.71, 732.27, 17654.54 and 8581.42 MJ/ha of energy for labour, seed, nitrogen, phosphorus, potassium, pesticide, irrigation water and fuel, respectively. The output gives an average yield of 2867.73 kg/ha of paddy equivalent to 53253.77 MJ of energy was obtained.

Level of energy consumption in rice production which is the total average energy used in various farm operations during rice production in the area was found to be 36,397.85 MJ/ha (Table 2). This is higher than the 23,358.75 MJ/ha reported by Iqbal (2007) in Bangladesh and 12906.8 MJ/ha reported by Ibrahim and Ibrahim (2012) in Nasarawa State in Nigeria. This might be due to the fact that most of the rice farmers were not efficient in their resource used in Jere Bowl (Malah, 2015). Of the total energy inputs consumed, 4.13% was from human labour, 7.36% seed, 14.41% fertilizers (the share of Nitrogen, Phosphorus and Potassium having 13.67, 0.46 and 0.28%, respectively), 2.06% pesticide (the share of insecticides and herbicides having 0.25 and 1.78%, respectively), and 48.51% irrigation water and 23.58% fuel (petrol energy was mainly consumed for irrigation water application) inputs. This indicates that the highest energy inputs were taken up by water and fuel. This is because irrigation operation consumed the maximum energy on the rice farm due to the higher water requirement of rice crop. Rice crop under irrigation was mostly grown with water from tube well and canal water. While the fuel (petrol) energy is mainly utilized by water pumps to run irrigation pump set. This shows the needs for efficient and cost effective irrigation system that will minimize the use of water resulting in less energy

Table 2. Level of energy consumption in rice production.

Input/Output	Quantity per unit area (Unit/ha)	Total energy equivalent (MJ/ha)	%
Human labour (h)	766.59	1502.51	4.13
Seed (kg)	153.00	2677.50	7.36
Fertilizer (kg)			
Nitrogen	82.07	4973.40	13.67
Phosphorus	15.18	168.51	0.46
Potassium	15.18	101.71	0.28
Agrochemical (L)			
Herbicide	2.28	646.55	1.78
Insecticide	0.43	85.72	0.24
Water for irrigation (M^3)	27817.76	17654.54	48.51
Fuel (L)	232.09	8581.42	23.58
Paddy (kg)			
Grain	2867.73	53253.77	-
Straw	2939.42	36742.80	-
Total Energy Input (Mj/ha)	-	36397.85	-
Total Energy output (Mj/ha**)**	-	89996.57	-

Source: Field Survey (2017)

Table 3. Forms of energy in rice production in Jere Bowl.

Energy forms	Measure (MJ/Ha)	Percentage
Direct energy	27738.48	76.21
Indirect Energy	8653.39	23.77
Renewable Energy	21834.55	59.98
Non-renewable energy	14557.31	39.99

Source: Field Survey (2017).

requirement.

Forms of energy in rice production

The different forms of energy according to direct, indirect, renewable and non-renewable energy are shown in Table 3. This table revealed that the ratio of direct energy (76.21%) to indirect energy (23.77%) is higher. This indicates that direct energy contribute much to rice production than the indirect energy. This is because water for irrigation and fuel are component of direct energy and both consumed high energy in the production. The table also showed that renewable energy constituted the higher energy (59.98%) while non-renewable constituted only 39.99% of energy to the production system. This implies that rice production system in the area causes less environmental pollution with minimum (39.99%) of non-renewable energy. This finding differs from that of Cherati et al. (2012) in the study of energy and economic analysis of three varieties of rice production in North Iran who reported higher non-renewable energy. This is because fertilizer and agrochemicals which are the major sources of non-renewable energy were not being used adequately by the farmers in Jere Bowl due to its cost and affordability by the farmers.

Energy indices of rice production

The energy indices of rice production estimated in this study were energy ratio, specific energy, energy productivity, water productivity, and net energy.

Energy ratio

Energy ratio is the ratio of total average energy input to

Table 4. Energy Indices of Rice Production in Jere Bowl.

Indices	Unit	Amount
Energy ratio	-	2.473
Specific energy	Mj/kg	31.383
Energy productivity	kg/Mj	0.147
Water productivity	kg/m^3	0.103
Net energy	Mj/Ha	53598.710

Source: Field Survey (2017)

total average energy output. Table 3 revealed that the energy ratio in Jere Bowl agro-ecosystems was 2.47. This implies that the farmers in Jere Bowl earn in terms of energy at least 2.47 times of what they put into the production process. This index is lower and could be as a result of inefficient use of some energy inputs due to inefficient irrigation system. This finding compares closely to Alipour et al. (2012) that rice energy ratio in Guilan province of Iran was 2.19 lower than 6.7 rice energy ratio index estimated in Australia by Khan et al. (2010). As rice farm practices in Jere Bowl in Nigeria and Guilan province in Iran are considerably not modernized and efficient as in Australia.

Specific energy

Specific energy is an index which shows how much energy was used to produce one unit of disposable/ marketable yield (rice grain). The lower the index the more efficient is the use of energy in the production system. The result revealed an index of 31.38 MJ/kg (Table 3), indicating that about 31.38 MJ of energy is required to produce only a kilogram of paddy. This implies that there was low grain output in respect to energy inputs used in the production process due to inefficient energy inputs used. This might be due to the inefficient practices of the rice farmers in their production in Jere Bowl as reported by Malah (2015).

Energy productivity

Energy productivity is the yield of marketable product, that is, rice grain per unit of energy consumed. The higher the value (>1), the more energy efficient is the production system. Table 4 shows that energy productivity of rice production in Jere Bowl was 0.147 kg/Mj indicating lower energy productivity. This implies that one Mj of energy used by the farmers produced only 0.147 kg of paddy rice. The lower energy relevance in the area could justify lower productivity on energy consumption in the area. This compares closely to the findings of Asmat (2009) that the rice energy productivity for small, medium and large farmers in Thailand were 0.32, 0.28 and 0.24 kg/Mj, respectively.

Water productivity

Water productivity is the ratio of rice grain yield to irrigation water consumed. The higher the value (>1) the more water productive is the production. Table 4 shows that the water productivity index in Jere Bowl was 0.103 kg/M^3 indicating that water productivity in the Jere Bowl was low. This is apparently because of inefficient irrigation system and ineffective water management. This index was much lower as compared to studies by Khan et al. (2009) in Pakistan with 0.33 kg/m^3 for rice crop.

Net energy

The amount of net energy calculated in the Jere Rice Bowl agro-ecosystems was approximately 53,598.71 Mj/ha (Table 4) which was considerably lower than the net energy of 86,050 MJ/ha reported in Bangladesh by Iqbal (2007). This observation could be argued by the statement that overusing of inputs caused increment in consumed energy and lower yield of rice in this region compared to other areas in the world. This is because of inappropriate management practices, planting of low yielding indigenous varieties and perhaps decreasing return to scale could clarify the low yield of rice in this area as well.

All the energy indices measured in Table 4, indicated inefficient use of all the energy inputs. This might affect the sustainability of irrigated rice production in the area because a step towards achieving sustainable production (that is, efficient use of energy inputs).

Conclusion

A quantitative energy input-output analysis of irrigated rice production in Jere Bowl Borno State, Nigeria was studied based on the level of energy consumption, forms of energy and some energy indices such as energy ratio, specific energy, energy productivity, water productivity and net energy. On an average, total energy input was

estimated as 36,397.85 MJ/ha. The highest energy inputs were taken by water (17654.54 MJ/ha) and fuel use (petrol) (8581.42 MJ/ha). This is because irrigation operation consumed the maximum energy on rice farm due to the higher water requirement of rice crop while the fuel energy is mainly utilized by water pumps to run irrigation pump set. The results on energy indices of rice production indicated 2.47, 31.38 MJ/kg, 0.147 kg/MJ, 0.103 kg/M^3 and 53,598.71 Mj/ha for energy ratio, specific energy, energy productivity, water productivity and net energy, respectively indicating lower values of energy indices due to inefficient use of energy inputs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Alipour A, Veisi H, Darijani F, Mirbagheri B, Behbahani AG (2012). Study and Determinationof Energy Consumption to Produce Conventional Rice of the Guilan Province. Research in Agricultural Engineering 58(3):99-106.

Ann HJ (2011). Calculation of the carbon footprint of Ontario Wheat. SURG Journal 4(2):49-55.

Asmat U (2009). A comparative analysis of energy use patterns in Small and large scale irrigated rice farming Systems: A Case Study in Ayutthaya Province in the Central Region of Thailand. (Unpublished Msc theses) Asian Institute of Technology School of Environment, Resources and Development Thailand. https://agritrop.cirad.fr/553489/1/document_553489.pdf

Cherati FE, Bahrami H, Asakereh A (2011). Energy Survey of Mechanized and Traditional Rice Production System in Mazandaran Province of Iran. African Journal of Agricultural Research 6(11):2565-2570.

Cherati FE, Kamyab S, Shekofteh M, Amraei A (2012). Investigation of energy and economic analysis of three varieties of rice production in North Iran. Research Journal of Applied Sciences, Engineering and Technology 4(16):2666-2671.

Erdal GK, Esengun OH, Gunduz O (2007). Energy use and economical analysis of sugar beet production in Tokat province of Turkey. Energy 32:35-41.

Falaye A (2013). Energy Input-Output, Optimization of Energy Consumption with DEA Approach for Corn Silage Production in Iran. International Journal of Agriculture and Crop Sciences 5(1):80-88.

Gevao SM, Ismail W, Yahya Y, Wan C (2007). Analysis of Energy Consumption in Lowland Rice-Based Cropping System of Malaysia. Energy 27(4):820.

Gundogmus E (2006). Energy use on Organic Farming: a Comparative Analysis on Organic versus Conventional Apricot Production on Small Holdings in Turkey. Energy conversion and management 47:3351-3359.

Ibrahim HY, Ibrahim HI (2012). Energy Use Analysis for Rice Production in Nasarawa State, Nigeria. Tropical and Subtropical Agroecosystems 15:649-655.

Inuwa IMS, KyiogwomAla AL, Maikasuwa MA, Ibrahim ND (2011). Profitability Analysis of Rice Processing and Marketing in Kano State, Nigeria. Nigerian Journal of Basic and Applied Sciences 34(18):2796-3801.

Iqbal MT (2007). Energy Input and Output for Production of Boro Rice in Bangladesh. EJEAFChe. 6(5):2144-2149.

Jibirin JM (2010). Forms of potassium and potassium absorption in some Fadama soil of Nigeria. Savannah Journal of Agriculture 5:1597-1613.

Kaltsas AM, Mamolos AP, Tsatsarelis CA, Nanos GD, Kalburtji KL (2007). Energy Budget in Organic and Conventional Olive Groves. Agriculture, ecosystems and environment 122:243-251.

Karale S, Khambalkar V, Bhende B, Sharddha A, Pranali W (2008). Energy Economic of Small Farming Crop Production Operations. World Journal of Agricultural Sciences 4(4):276-482.

Khan MA, Awan IU, Zafar J (2009). Energy Requirement and Economic Analysis of Rice Production in Western part of Pakistan. Soil and Environment 28(1):60-67.

Khan S, Khan MA, Latif N (2010). Energy Requirements and Economic Analysis of Wheat, Rice and Barley Production in Australia. J. Soil Environ. 29(1):61-68.

Malah JB (2015). Technical Efficiency of Rice Producers at the Jere Bowl of Jere Local Government Area of Borno State, Nigeria. Unpublished Msc thesis department of agricultural economics and extension services. University of Maiduguri, Borno state, Nigeria.

National Population Census (NPC) (2006). Population Census Data Borno State, Nigeria Federal Republic of Nigeria Official Gazette, National and State Provisional Totals Census. Printed and Published in 2007 by the Federal Government Printer, Lagos, Nigeria. 94(21):175-198.

Nigerian Metrological Agency (NMA) (2008). Annual Report. Office Memo File. http://www.nimet.gov.ng/nwp

Nyanganji JK (1994). The Morphology and Hydrography of the Ngadda Catchments and the Bama Beach Ridge" Unpublished PhD Thesis, Bayero University Kano.

Singh H, Singh AK, Kushwaha HL, Singh A (2007). Energy consumption pattern of wheat production in India. Energy 32:1848-1854.

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Screening of local and improved bean varieties for resistance to halo blight disease

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The study was conducted to screen local and improved bean varieties for resistance to halo blight disease caused by *Pseudomonas syringae* pv. *phaseolicola*. A total of eight improved (Sokoine University of Agriculture (SUA) 90, Rojo, Zawadi, Mshindi) and local (Mwasipenjele, Masusu, Kabhaya, Mabula) bean varieties were collected from breeders at SUA and farmers at Ileje district in Songwe region. Isolation of *P. syringae* pv *phaseolicola* from bean seeds was conducted using Liquid Assay method. Results indicated that, bacterial isolate L1 from local bean variety Mwikala produced similar characteristics as those obtained from reference strain of *P. syringae* pv *phaseolicola* in the biochemical and pathogenicity tests on host plants. Using bacterial isolate L1, there was significance difference ($P < 0.05$) on incidence and severity of halo blight disease. The highest disease incidence had (89%) was on local bean variety Mabula, while the lowest had (67%) was on improved varieties Zawadi and Mshindi. Disease severity of improved varieties zawadi and mshindi when compared with other varieties were at low severity (disease score 4). It was concluded that the Zawadi and Mshindi were less susceptible to halo blight disease. This study needs to be repeated if same results were obtained aside these two improved varieties (Zawadi and Mshindi) of bean which could be recommended to farmers.

Key words: Assay, incidence, inoculum, isolate, *Pseudomonas syringae* pv. *phaseolicola*, disease severity.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important leguminous crop and native to America (Gepts and Dpbouk, 1991). It is an important source of minerals, protein content (~22%) and vitamins for many developing countries where they cannot afford meat (Moraghan and Grafton, 2001; Hillocks et al., 2006; Graham et al., 2007;). Common bean complements cereals and other carbohydrate rich foods in providing near perfect nutrition to people of all ages and helps to lower cholesterol and cancer risks (Singh, 1999). The major producers of common bean worldwide are Brazil, Mexico, United State, Ethiopia, Turkey, Indonesia, Tanzania, Uganda and Angola (Beebe et al., 2013). In Africa, bean production is concentrated in densely populated Eastern and Southern Highlands of the continent (Beebe et al., 2000). In Tanzania, bean is grown in cool regions, particularly the Southern Highlands (Iringa, Songwe, Mbeya, Rukwa, Katavi, Njombe and Ruvuma regions), Northern Highlands (Arusha, Tanga, Kilimanjaro and Manyara), Western Highlands (Kagera and Kigoma)

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and in Central Morogoro especially in areas surrounding Uluguru Mountains (Mabagala et al., 2000). Over 75% of rural households in Tanzania depend on common beans for daily sustenance (CIAT, 2008). Consumption of common bean is high because it is relatively inexpensive as compared to meat (Pachico, 1989; Fivawo and Msolla, 2011). However, its production is constrained by a number of factors including diseases especially halo blight caused by *Pseudomonas syringae* pv *phaseolicola* (Tock et al., 2017). Halo blight is a bacterial disease caused by *P. syringae pv phaseolicola*. It is a serious seed-borne disease of common bean worldwide (Popovi et al., 2012; Boersma et al., 2014; Chatterton et al., 2016). Halo blight disease of beans cause low yield of about 37% in many places of the world especially in the developing countries (Taylor et al., 1996a, b; Gondwe, 1998; Chataika et al., 2011; Duncan et al., 2014). Halo blight is difficult to control. Pathogen free seeds can be used to control the spreading of the disease however; it does not guarantee disease control as other inoculum sources exist (Fourie, 2011). The disease can be controlled by using new improved resistant varieties (Tock et al., 2017). Cultural practices such as removing, destroying or deep ploughing of debris, effective weed control, crop rotation and minimizing movement within fields when foliage is wet, may reduce the disease (Mbega et al., 2012). However, these cultural practices do not eliminate the disease especially when environmental conditions are favourable for disease development. Chemical control is considered effective but most chemicals fail to treat disease due to resistant strains and improper application by farmers which may cause environmental pollution (Mbega et al., 2012). Host plant resistance is considered the most effective control strategy for halo blight (Fourie, 2011). Therefore, this study aimed to evaluate improved and local bean varieties for resistance to halo blight under greenhouse condition. The results can be used for breeding purposes against this disease in Morogoro, Tanzania.

MATERIALS AND METHODS

Location of the study area

The research was conducted at the AfSHC Laboratory at SUA in 2016/2017. The university is located at 3 km from the Centre of Morogoro Municipality, which is about 200 km west of Dar es Salaam. It has an altitude of 526 m and lays at latitude 6°19'S and longitude 37°40'E.

Survey and seed collection

The survey was conducted in Morogoro and Songwe regions. Improved bean seed sample varieties (Mshindi, Zawadi, SUA 90 and Rojo) were obtained from plant breeders at SUA in Morogoro region. Four local bean sample varieties (Masusu, Mwasipenjele, Kabhaya and Mwasipenjele) were collected from randomly selected farmers at Ileje-Songwe. Seed samples (1 kg) were packed in paper bags and labelled appropriately: collection date, date of harvest, name of collector, variety and location. The samples were transported to the AfSHC Laboratory for further analysis.

Working seed samples

The working seed samples were obtained by the following International Seed Testing Association rules (ISTA, 2005). The conical divider was cleaned with compressed air before seeds were divided. The seed sample was poured over the hopper then valve was released and locked. In this procedure, the sample was divided into two halves and was collected in the two collecting pans. The seeds were removed from one of the pans into a container and poured back into the original seed bag and the seeds from the other pan were poured again into the hopper and the procedure was repeated until a working sample (700 g) was obtained as described by Mathur and Kongsdal (2003).

Determination of purity, moisture content and seed germination

Purity analysis

The purity of seed samples was determined using ISTA rules (ISTA, 2005). Each sub sample was analysed into the following categories: pure seed, inert matter and seed of the other crops and the results were expressed in percentages.

Determination of moisture content (MC)

The lower constant temperature oven method was used to determine the moisture content of bean seeds (Anonymous, 1999). In this method, 10 g of seed was randomly picked from the original submitted sample and were evenly distributed over the surface of 8.5 cm diameter container (Petri dish). The container and its cover were weighed before and after filling it with the seeds. The containers were placed in an oven set at 103±2°C and dried for 17 h. The moisture content determination was carried out using two independently drawn working samples. After drying the seed in the oven, the container was covered and cooled for 30 to 40 min. The moisture content percentage was calculated using the formula: $MC= (M_2-M_3) \times 100/M_2-M_3$; where M_1 was weight in grams of the container with its cover; M_2 was weight in grams of the container, cover and the content before drying. M_3 was weight in grams of the container, cover and the content after drying.

Isolation of bacteria from bean seed samples

Liquid assay was used to detect *P. syringae* pv. *phaseolicola* from bean samples. About 300 g of bean were tested using procedures described by Mortensen (2005). Seeds were washed three times in sterile distilled water put in transparent plastic bags containing 620 ml of sterile distilled water based on the formula (1.9 × weight of the seeds) + 50 described by Mortensen (2005). The samples were incubated at room temperature for 3 h. The 1 ml of the suspension was drawn from the sample and serially diluted to 10^{-3}. The samples were plated on Nutrient Agar (NA) plates at 28°C for 24 to 48 h. The colonies with morphology similar to *P. syringae* pv. *phaseolicola* reference strains were purified on NA and further tested as described under biological test.

Biochemical tests

The isolates from bean samples were tested for Gram reaction

using Potassium Hydroxide solution as described by Mortensen (2005). Only Gram-negative isolates that had colony morphology and colour similar to the reference bacterial strain of *P. syringae* pv. *phaseolicola* were selected for further tests as described under pathogenicity test.

Pathogenicity tests

Gram-negative isolates as previously described were tested for their pathogenicity on host bean variety Kabhaya. Pathogenicity test was conducted as described by Mortensen (2005). Bean seedlings grown in plastic pots (16 cm diameter × 13 cm height) containing forest soil at 2 leaf stage were spray-inoculated with a bacterial suspension of ca.ca. 10^8 cfu/ml from bacterial isolates and reference bacteria strain (*P. syringae* pv. *phaseolicola*). Plants sprayed with sterile distilled water alone served as a negative control. Isolates which produced water-soaked symptoms as those produced on plants sprayed with a *P. syringae* pv. *phaseolicola* were selected for further tests.

Screening bean varieties for resistance to halo blight

Seed inoculation with bacteria

Eighteen seeds from each bean variety previously described were pre-germinated using between paper method (ISTA, 2005) and after three days, the germinating seedlings were inoculated by stabbing the cotyledons with a needle smeared with bacteria suspension of ca. 10^8 cfu/mL isolated from variety Mwikala collected from Ileje, Songwe. In addition, seeds inoculated with a reference bacterial strain *P. syringae* pv. *phaseolicola* (obtained from the AfSHC) and sterile distilled water were used as controls (Mortensen, 2005). The seedlings were further evaluated as described under greenhouse experiment.

Greenhouse experiment

Complete randomized design (CRD) with three replications was used in the greenhouse to test eight bean varieties for their resistance to Halo blight disease. Three seedlings previously inoculated with the bacteria and controls (in three replications) were planted in sterile forest soil in a screen house at 25 to 28°C with relative humidity of 70 to 80% for 56 days. Data were recorded in one week interval for six weeks.

Determination of disease incidence

The disease incidence was calculated by dividing total number of infected plants by total number bean plants by 100%.

Disease severity

Halo blight disease was evaluated by visual observation using a 1 to 9 scale (Schoonhoven and Pastor-Corrales, 1987), where 1= no disease symptoms, >1- 3= slightly disease symptoms, >3-6= moderate disease symptoms and >6- 9= severe symptoms to death of plants.

Data analysis

The data was analysed at the significance level of 5% using GEN START-software. The significance difference between bean varieties was computed using Analysis of Variance (ANOVA) and the difference between means was established using Duncan's Multiple Range Test (Kothari, 2004).

RESULTS

Purity of bean seed samples used in this study

The results for purity analysis indicated that bean seed sample number 3 variety Rojo collected from SUA had the highest (99.98%) proportion of pure seed followed by sample number 2 (99.97%), 1 (99.93%), 6 (99.90%), 4 (99.89%), 7 (99.67%), and 5 (99.16%) (Table 1). Bean seed sample number 8 variety Mabula had the lowest (96.37%) proportion of pure seed.

Moisture content of bean seed samples used in this study

The results showed that the moisture content determined using lower constant temperature oven method of bean seed samples ranged from 10 to 14.5% (Table 1). Bean variety Kabhaya collected from Ileje had the highest moisture content (14.5%). The lowest moisture content (10%) was obtained from bean variety Mwasipenjele collected from Ileje, Songwe (Table 1).

Seed germination

The results of germination test conducted using blotter paper method indicated that all seed samples had 100% of germination (Table 1). Such results demonstrated that, bean seed samples whether local or improved used in this study had a good germination.

Bacterial isolates obtained from bean seed samples

Using liquid assays to isolate bacteria from bean seed samples, a total of three isolates (Table 2) with similar morphological characteristics like the reference *P. syringae* pv. *phaseolicola* were obtained from variety Mwikala. No bacterial isolates resembling the reference strain (*P. syringae* pv. *phaseolicola*) was obtained from other bean seed samples used in this study.

Gram reaction of the isolates

The gram reaction test using 3% potassium hydroxide solution showed that, all three isolates resembled the colon colour and morphology of reference bacterial strain of *P. syringae* pv. *phaseolicola* were Gram-negative (Table 2). The results of pathogenicity on host bean plants as described by Mortensen (2005) indicated that

Table 1. Purity, moisture content and germination of bean seeds used in this study.

Sample No.	Variety	SG	Location	PS	IS	OSC	MC (%)	GM (%)
1	SUA 90	Improved	SUA	0.01	0.01	0	12.5	100
2	Mshindi	Improved	SUA	99.97	0.01	0	13	100
3	Rojo	Improved	SUA	99.98	0.03	0	14	100
4	Zawadi	Improved	SUA	99.89	0.01	0.03	11.5	100
5	Mwikala	Local	Ileje	99.16	0.4	0	12.4	100
6	Kabhaya	Local	Ileje	99.9	0.9	0	14.5	100
7	Mwasipenjele	Local	Ileje	99.67	0.03	0	10	100
8	Mabula	Local	Ileje	96.37	3.34	0	12	100

SG: Seed grade, PS: pure seed, IS: inert seed, OSC: other seed, MC: moisture content, GM: germination.

Table 2. Gram reaction and pathogenicity of bacteria isolates obtained from variety Mwikala.

Isolate	Bean variety	Gram reaction	Pathogenicity tests of isolates on variety Kabhaya
L1	Mwikala	−ve	+
L2	Mwikala	−ve	+
L3	Mwikala	−ve	+
*P.s.*pv. *phaseolicola*	AfSHC	−ve	+
Steriledistilled water	N/A	N/A	N/A

N/A: Not applicable, -ve Gram negative reaction, + reaction for pathogenicity test and - negative reaction for pathogenicity test.

bean seedlings sprayed with the Gram negative bacterial isolated from bean sample variety Mwikala caused water-soaked symptoms as those produced in seedlings sprayed with the reference bacteria strain (*P. syringae* pv. *phaseolicola*) (Table 2). Thus, since these isolates were obtained from one bean seed sample, only one isolate (isolate L1) was selected and used in the screening of bean varieties for their resistance to halo blight.

Halo blight disease incidence on bean cotyledons and leaves inoculated with bacteria

In the greenhouse experiment, the results showed that bean cotyledon inoculated with bacterial isolate L1 and the reference bacterial strain *P. syringae* pv. *phaseolicola* produced disease symptoms and had higher disease incidence than bean cotyledons inoculated with sterile distilled water (Table 3). Disease symptoms were not observed in bean cotyledons inoculated with sterile distilled water control. Using isolate L1, bean sample number 8 (variety Mabula) collected from Ileje had the highest (89%) disease incidence on cotyledon when compared with other bean samples (Table 3). Bean sample numbers 4 (variety Zawadi) and 7 (variety Mwasipenjele) had the lowest (67%) disease incidence (Table 3). The disease incidence of other samples inoculated with the bacteria cultures is shown in Table 3. These results indicated that these two bean varieties were less susceptible to halo blight disease than other bean varieties screened. Such results also demonstrated that bean cultivars Zawadi and Mwasipenjele were resistant to halo blight disease compared with other bean samples screened.

Disease severity on cotyledons and leaves of bean inoculated with bacterial isolates

Using a scale of 1 to 9 to determine the severity of halo blight on bean cotyledon and leaves, the results showed that, there was significance difference ($P<0.05$) among bean varieties assessed. Bean samples inoculated with isolate L1 and the reference strain *P. syringae* pv. *phaseolicola* had high disease severity of 4 up to 7 in different varieties (Table 4). All plants inoculated with sterile distilled water had a disease score of 1, indicating no disease (Table 4). Of the plant tested, bean sample variety Mabula together with bean varieties SUA 90, Rojo and Mwikala had the highest (disease score of 7) disease severity both in the test isolate L1 and reference strain *P. syringae* pv. *phaseolicola*. Bean varieties Zawadi and Mshindi had the lowest (disease score of 4) on cotyledons inoculated with either isolate L1 or reference strain *P. syringae* pv. *phaseolicola* (Table 4). These results demonstrated that, bean cultivars Zawadi and Mshindi were less susceptible to halo blight when

Table 3. Halo blight disease incidence on cotyledons and leaves of bean varieties.

Bean variety	Incidence of halo blight			Bean variety	Incidence of halo blight		
	Cotyledons (%)		SDW		Leaves (%)		SDW
	L1	P.s.pv.p			L1	P.s.pv.p	
Zawadi	67^{a}	65^{a}	1^{a}	Zawadi	1^{a}	0^{a}	1^{a}
Mshindi	67^{a}	73^{ab}	1^{a}	Mshindi	1^{a}	0^{a}	1^{a}
Mwasipenjele	67^{a}	76^{ab}	1^{a}	Mwasipenjele	1^{a}	0^{a}	1^{a}
Kabhaya	78^{ab}	74^{ab}	1^{a}	Kabhaya	1^{a}	0^{a}	1^{a}
Mwikala	78^{ab}	77^{ab}	1^{a}	Mwikala	1^{a}	0^{a}	1^{a}
Rojo	78^{ab}	78^{ab}	1^{a}	Rojo	1^{a}	3^{ab}	1^{a}
SUA 90	78^{ab}	83^{b}	1^{a}	Mabula	3^{ab}	12^{b}	1^{a}
Mabula	89^{b}	91^{b}	1^{a}	SUA 90	3^{ab}	13^{b}	1^{a}

L1: Bacterial isolated from bean variety Mwikala, *P.s*.pv.*p*: *Pseudomonas syringae* pv *phaseolicola*, SDW: sterile distilled water. Means followed by the same letters in a column are not significantly different.

Table 4. Halo blight disease (1-9 scale) severity of cotyledons and leaves of bean varieties.

Bean variety	Severity of halo blight on			Bean variety	Severity of halo blight		
	Cotyledons (%)		SDW		Leaves (%)		SDW
	L1	P.s.pv.p			L1	P.s.pv.p	
Zawadi	4^{a}	4^{a}	1^{a}	Kabhaya	1^{a}	1^{a}	1^{a}
Mshindi	4^{a}	4^{a}	1^{a}	Mshindi	1^{a}	1^{a}	1^{a}
Mwasipenjele	4^{a}	4^{a}	1^{a}	Mwasipenjele	1^{a}	1^{a}	1^{a}
Kabhaya	7^{ab}	7^{ab}	1^{a}	Zawadi	1^{a}	1^{a}	1^{a}
Mwikala	7^{ab}	7^{ab}	1^{a}	Mwikala	1^{a}	1^{a}	1^{a}
Rojo	7^{ab}	7^{ab}	1^{a}	Rojo	1^{a}	1^{a}	1^{a}
SUA 90	7^{ab}	7^{ab}	1^{a}	SUA 90	3^{ab}	3^{ab}	1^{a}
Mabula	7^{ab}	7^{ab}	1^{a}	Mabula	3^{ab}	3^{ab}	1^{a}

L1: Bacterial isolated from bean variety Mwikala, *P.s*.pv.*p*: *Pseudomonas syringae* pv *phaseolicola*, SDW: sterile distilled water. Means followed by the same letters in a column are not significantly different.

compared with other bean varieties used in this study (Table 4).

DISCUSSION

Based on the results of this study the low proportion of pure seed was attributed to by contamination by inert matter as indicated by the proportion of as high as 3.34% (Table 1). Presence of high proportion of inert matter in bean samples may be caused by poor threshing and winnowing which lower the quality of seed. Purity of bean seed samples show that these bean samples had purity that met minimum requirements (98%) recommended by Food and Agriculture Organization (FAO) (FAO, 2011). On the other hand, variation in moisture contents of the seeds observed is accelerated by improper drying of the seed and/or poor storage rooms (Nahar et al., 2009). The results indicated significance differences of disease incidence and severity among bean varieties tested which is in agreement with previous studies which report different races of halo blight affecting common bean (Félix-Gastélum et al., 2016; Tock et al., 2017). The resistance common bean varieties for race 6 are available in East Africa. Resistance varieties may be introgressed into susceptible varieties using marker assisted backcrossing for production of new resistance varieties (Chataika et al., 2011). The absence of disease severity on plants inoculated with sterile distilled water was due to unavailability of causal pathogen (*P. syringae* pv. *phaseolicola*). For effective screening for resistance to halo blight, *P. syringae* pv. *phaseolicola* isolates should be originated from one among varieties tested, this improve effectiveness of screening and correct selection of resistance varieties to halo blight (Murillo et al., 2010). Application of bio-control concentration of *P. syringae* pv. *phaseolicola* isolates show variation of susceptibility to common bean varieties, this is an important approach for resistance common bean to the disease (Eman and Afafa, 2014). High disease incidence and severity on cotyledons for varieties (SUA 90, Rojo, Kabhaya, Mabula) and leaves for varieties (SUA 90, Rojo

and Mabula) to halo blight could be influenced by poor genetic resistance of beans to halo blight races. Similar results were reported by Asensio et al. (2010) who conducted field evaluations under inoculated conditions and identified two accessions (BGE 002189 and BGE 029592) out of 199 from a Spanish core collection that had immune reaction to two races of *P syringae* pv. *phaseolicola* (races 6 and 7). Previous study by Küçük et al. (2016) reported the same trend of common bean susceptibility to halo blight disease. Nevertheless, field evaluation is recommended to confirm greenhouse results. Legume species have been infected by at least nine races of halo blight of which the races number 1, 2 and 6 are common worldwide (Taylor et al., 1996a, b), while races number 3, 4, 5 and 8 are common in the Eastern and Southern of Africa. Furthermore, races 5 and 8 are dominant in Africa (Teverson and Taylor, 1994). Up to 43% reductions in total yield have been reported and further losses occur owing to the poor quality and high incidence and severity of halo blight which infected pods, seeds and leaves (Dawn et al., 2010). In Tanzania, races 1 (45%), 2 (52%) and 3 (3%) have been reported, therefore, Tanzania is among the African countries which are affected by the disease; this correspond to our results of which most varieties were susceptible to the disease (Mabagala and Saettler, 1992). The varieties Zawadi and Mshindi which were resistant to halo blight probably could have genes cv or Quantitative Trait Loci (QTLs) associated with gene cv. QTLs have significance contribution to the expression of target trait under phenotypic evaluation. Therefore, QTL mapping for halo blight resistance in common bean is essential to identify the superior varieties (González et al., 2017). Red Mexican which is resistant to race 1 and PI 150414 for races 1 and 2 that have been useful in breeding for halo blight resistance (Hillocks et al., 2006). Lines with I-gene were also reported to be highly resistant to race 3 of halo blight in Burundi (Schmit, 1994). Our results indicated variation of bean varieties for susceptibility to halo blight where Zawadi and Mshindi had the lowest compared to other bean varieties. Previous study by Fivawo and Msolla (2011) has reported comparable results of which variation of susceptibility of halo blight disease on bean varieties accounted to exist. The variation of susceptibility can be influenced by several factors such as gene sequence, strength of leaves to resist and source of origin. By introducing new genes with resistance to a wide range of halo blight races infecting legumes will contribute to enhance high production of beans (Fourie, 2011). Based on our study, the varieties which showed high susceptibility to halo blight would be infected by the races which have been reported before. The genes sequence for resistance need be identified by sequencing technology for breeding improvement programmes (Fourie et al., 2004; Teverson, 1991; Taylor et al., 1996b). The varieties with less susceptible to halo blight therefore, is important in plant breeding and should be promoted to farmers for cultivation (Allen et al., 1998; Fourie, 2011).

Conclusion

It was concluded from this study that, bacterial isolate (LI) obtained from local bean variety Mwikala produced similar characteristics as those obtained with the reference strain of *P. syringae* pv. *phaseolicola* on the biochemical and pathogenicity tests on host plants. Such results indicated that his bean sample was infected by *P. syringae* pv. *phaseolicola*. By inoculating bacterial isolate L1 on different bean samples used in this study, the incidence of halo blight disease was the highest (89%) on the cotyledons of local bean variety Mabula collected from Ileje, Songwe followed by Mwikala (78%), SUA 90 (78%), Rojo (78%), Kabhaya (78%), Zawadi (67%) and Mshindi (67%). Disease symptoms were not observed in bean seedlings inoculated sterile distilled water. Such results indicated, the improved varieties Zawadi and Mshindi were less susceptible to halo blight disease as compared to other bean varieties used in this study. Further evaluation of disease severity indicated low severity (disease score of 4) on improved varieties Zawadi and Mshindi when compared with other bean varieties. Therefore, further evaluation was recommended (using large number of samples and in different environmental conditions) of bean varieties Zawadi and Mshindi so that if similar results will be obtained, then the two varieties can be promoted to farmers in Morogoro and Songwe regions as well as the source of breeding materials for improvement of bean in Tanzania.

CONFLICT OF INTERESTS

The author declared no conflict of interest in this paper.

REFERENCES

Allen DJ, Buruchara RA, Smithson JB (1998). Diseases of common bean. In The Pathology of food and pasture legumes (D.J. Allen & J.M. Lenne, eds):. CAB International, Wallingford pp. 179-235.

Anonymous. (1999). International Rules for Seed Testing. International Seed Testing Association. Seed Science and Technology 27:47-50.

Asensio C, Asensio S-Manzanera MC, Ibeas A, de la Rosa L (2010). Resistance to halo blight, common bacterial blight, and bacterial brown spot in Spanish common bean core collection. Annual Report. Bean Improvement Cooperative 53:110-111.

Beebe S, Skroch PW, Tohme J, Duque MC, Pedraza F, Nienhuis J (2000). Structure of genetic diversity among common bean landraces of Mesoamerican origin based on correspondence analysis of RAPD. Crop Science 40:264-273.

Beebe SE, Rao IM, Blair MW, Acosta-Gallegos JA (2013). Phenotyping common beans for adaptation to drought. Frontiers in Physiology, 4(2):35.

Boersma JG, Conner RL, Balasubramanian PM, Navabi A, Yu K, Hou A (2014). Combining resistance to common bacterial blight, anthracnose, and bean common mosaic virus into Manitoba-adapted

dry bean (*Phaseolus vulgaris* L.) cultivars. Canadian Journal of Plant Science 94:405-415.
Chataika BYE, Bokosi JM, Chirwa RM1, Kwapata MB (2011). Inheritance of halo blight resistance in common bean. African Crop Science Journal 19(4):325-333.
Chatterton S, Balasubramanian PM, Ericlson RS, Hou A, Mclaren DL, Henriquez MA, Conner RL (2016). Identification of bacterial pathogens and races of Pseudomanas syringae pv. phaseolicola from dry bean fields in western Canada. Canadian Journal of Plant Pathology 38:41-54
CIAT (2008). The impact of improved bean production technologies in Northern Tanzanaia. http://www.ciat.cgiar.org/work/Africa/Documents/highlight42.pdf.
Dawn L, Arnold D, Helen C, Lovell HC, Robert W, Jackson RW, Mansfield JW (2010). *Pseudomonas syringae* pv. *phaseolicola*: from 'has been' to supermodel. Molecular Plant Pathology, DOI: 10.1111/J.1364-3703.2010.00697.X
Duncan RW, Gilbertson RL, Lema M, Singh SP (2014). Inheritance of resistance to the widely distributed race 6 of Pseudomonas syringae pv. phaseolicola incommon bean pinto US14HBR6. Canadian Journal of Plant Science 94:923-928.
Eman OH, Afafa ZAE (2014). Biocontrol of Halo blight of Bean caused by *Pseudomonas phaseolicola*. International Journal of Virology 10(3):235-242
Félix-Gastélum R, Maldonado-Mendoza IE, Navarrete-Maya R, Olivas-Peraza NG, Brito-Vega H, Acosta-Gallegos JA (2016). Identification of Pseudomonas syringae pv. phaseolicola as the causal agent of halo blight in yellow beans in northern Sinaloa, Mexico. Phytoparasitica 44:369-378.
Fivawo NC, Msolla SN (2011). The diversity of common bean landraces in Tanzania. Tanzania Journal of Natural Applied Sciences 2(1):337-351.
Fourie D (2011). Susceptibility of South African dry bean cultivars to bacterial diseases. African Crop Science Journal, 19(4):387-392.
Fourie D, Miklas P, Ariyarathne HM (2004). Genes conditioning resistance to halo blight occur in a tight cluster. Annual Report. Bean Improvement Cooperative 47:103-104.
Gepts P, Dpbouk D (1991). Origin, domestication, and evolution of the common bean (Phaseolus vulgaris L.). Common beans: Research for Crop Improvement, Van Schoonhoven A and Voyset O (Eds), Wallingford, England. CAB International pp. 7-53.
Gondwe BJ (1998). Ecology, Epidemiology and Pathogenic variability *of Psedomonas syringae pv, phaseolicola* in the southem highlands of Tanzania. PhD Thesis. SUA Morogoro, Tanzania 16I p.
González AM, Godoy L, Santalla M (2017). Dissection of Resistance Genes to Pseudomonas syringae pv. Phaseolicola in UI3 Common Bean Cultivar. International Journal of Molecular Sciences 18:2503.
Graham RD, Welch RM, Saunders DA, Ortiz-Monasterio I, Bouis HE, Bonierbale M, Haan S, Burgos G, Thiele G, Liria R, Meisner CA, Beebe SE, Potts MJ, Kadian M, Hobbs PR, Gupta RK, Twomlow S (2007). Nutritious subsistence food systems. Advances in Agronomy 92:2-75.
Hillocks RJ, Madata CS, Chirwa R, Minja, EM, Msolla S (2006). *Phaseolus* bean improvement in Tanzania, 1959–2005. Euphytica DOI: 10.1007/s10681-006-9112-9
International Seed Testing Association (ISTA) (2005). International rules for seed testing. International Seed Testing Association (ISTA), Bassersdorf CH-Switzerland.
Kothari CR (2004). Research methodology: Methods and Techniques; New Age International (P) Ltd, Publishers.
Küçük C, Cevheri C, Özçelik H, Ertekin DC (2016). Determination of resistance of bean genotypes/lines to halo blight disease. Jokull Journal 66:6.
Mabagala RB, Mortensen CN, Mathur SB (2000). Halo blight, common and fuscous blights of bean with special reference to Tanzania. Field Inspection and Certification Procedures pp. 7-12.
Mabagala RB, Saettler AW (1992). The role of weeds in survival of *Pseudomonas syringae* pv. *phaseolicola* in Northern Tanzania. Plant Disease 76:683-687.
Mathur SB, Kongsdal O (2003). Common laboratory seed health testing methods for detecting fungi. *ISTA*,Switzerland.
Mbega ER, Mortenson CN, Mabagala RB, Wulf EG (2012). The effect of plant extracts as seed treatments to control bacterial leaf spot of tomato in Tanzania. Journal of General Plant Pathology 78:277–286.
Moraghan JT, Grafton K (2001). Genetic diversity and mineral composition of common bean seed. Journal of the Science of Food and Agriculture 81:404-408.
Mortensen CN (2005). Detection of *Xanthomonas oryzae* pv. *oryzae* from rice seeds by liquid assay. In: Seed Health Testing for Bacterial Pathogens. Danish Seed Health Centre for developing countries, Thorvaldsensvej 40, DK-1871, Frederiksberg C, Copenhagen, Denmark pp. 65-68.
Murillo J, Bardaji L, Führer E (2010). La grasa de las judías, causada por la bacteria *Pseudomonas syringae* pv. *phaseolicola*. Phytoma 224:27-32.
Nahar K, Ali MH, Ruhul AKM, Hasanuzzama M (2009). Moisture Content and Germination of Bean (*Phaseolus vulgaris* L.) Under Different Storage Conditions. Academic Journal of Plant Sciences 2(4):237-241.
Pachico D (1989). Trends in World common bean production problems in the Tropics. CIAT, Calli, Colombia pp. 1- 8.
Popovi T, Milovanovi P, Aleksi G, Gavrilovi V, Starovi M, Vasi M, Balaž J (2012). Application of semi-selective mediums in routine diagnostic testing of *Pseudomonas savastanoi*. Scientia Agricola 69(4):265-270
Schmit V (1994). Halo blight resistant varieties. CIAT Afiican Workshop Series 34:55-64.
Schoonhoven A, Pastor-Corrales MA (1987). Standard System for the Evaluation of Bean Germplasm. CIAT, Cali, Colombia 54:30.
Schwartz HF (1980). Miscellaneous bacterial diseases. in: Bean Production Problems. H. F. Schwartz, and G. E. Galvez, eds. Centro Internacional de Agricultura Tropical (CIAT), Apartado Aereo 6713, Cali, Columbia. pp. 173-194.
Singh SP (1999). Developments in plant breeding: Common bean improvement in the twenty-first century. Kluwer Academic Publishers, The Netherlands 409 p.
Taylor JD, Teverson DM, Davis JHC (1996b). Sources of resistance to *Pseudomonas syringae* pv. *phaseolicola* races in *Phaseolus vulgaris*. Plant Pathology 45:479-485.
Taylor JD, Teverson DM, Allen MA, Pastor-Corrales MA (1996a). Identification and origin of races of *Pseudomonas syringae* pv. *phaseolicola* from Africa and other bean growing areas. Plant Pathology 45:469-478.
Teverson DM (1991). Genetics of pathogenicity and resistance in the halo blight disease of bean in Africa. PhD. Dissertation, University of Birmingham, Birmingham, UK.
Teverson DM, Taylor JD (1994). Race characterization and identification of resistance to halo blight in Africa. CIAT African Workshop Series 34:44-54.
Tock AJ, Fourie D, Walley PG, Holub EB, Alvaro Soler A, Cichy KA, Pastor-Corrales MA, Song Q, Porch TG, Hart JP, Vasconcellos RCC, Vicente JG, Barker GC, Miklas PN (2017). Genome-Wide Linkage and Association Mapping of Halo Blight Resistance in Common Bean to Race 6 of the Globally Important Bacterial Pathogen. Frontiers in Plant Science 8:1170.

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