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Full Length Research Paper

Physiological response of soybean [*Glycine max* (L) Merrill] to soil moisture stress

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This study was done to determine the effects of varying soil moisture regimes on CO_2 assimilation of soybean [*Glycine max* (L.) Merrill] in pots under greenhouse conditions during 2017 and 2018 cropping seasons. The experiment was conducted as a Randomized Complete Block Design (RCBD) in a 4 x 6 factorial treatment arrangement and replicated 3 times. Soil moisture regimes (80, 60, 40 and 20% of field capacity) and cultivars (Gazelle, Nyala, EAI 3600, DPSB 8, Hill and DPSB 19) were first and second factors, respectively. Collected data were subjected to Analysis of Variance (ANOVA) using Linear Mixed Model in GENSTAT. Significantly different treatment means were separated using Tukey's test at 0.05 significance level. Leaf relative water content, stomata conductance, photosynthesis rate and sub-stomatal CO_2 concentrations significantly ($P < 0.001$) declined with increasing soil moisture stress. Total leaf chlorophyll content increased ($P < 0.001$) with increased soil moisture stress. Cultivars DPSB 19 and DPSB 8 had relatively higher leaf relative water content and stomata conductance at reduced soil moisture regime at 20% moisture from field capacity indicating moisture stress tolerance potential of the cultivars.

Key words: Flowering stage, podding stage, seasons, soil moisture regimes, soybean cultivars**.**

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is one of the most important legume crops with total production of 261.6 million metric tonnes worldwide (FAOSTAT, 2013). Soybean is a main source of protein, carbohydrates, vegetable oils, vitamins and minerals for human consumption and production of livestock feed. Soybean farming is also the most cost-effective ways resource-constrained smallholder farmers can use to maintain soil fertility of their lands as soybean helps to improve soil fertility through biological nitrogen fixation of soybean between 44 and 103 kg N ha^{-1} (Kananji et al., 2013; Ciampitti and Salvagiotti, 2018). The potential of soybean to significantly contribute to food and nutrition security and to generate substantial income for farmers is however constrained by low yields arising from soil moisture stress effects amongst other biotic and abiotic stresses. Soil moisture stress has become a recurring event due to unpredictable weather patterns arising from changes in climatic conditions occasioned by global warming (Abedinpour, 2012). Understanding the response of soybean to limited soil moisture stress, identification and use of moisture stress tolerant cultivars

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are options to reduce negative impacts of moisture stress and hasten soybean yield improvement (Farooq et al., 2009; Yunusa et al., 2014). This is more important considering that $^2/_3$ of global food production is through cultivation under moisture stress conditions (Madhu and Hatfield, 2015). Equally challenging to agriculture sector is the need to increase current food production levels by between 70 to 100% by the year 2050 in order to meet food requirements of the ever increasing human population (Alexandratos and Bruinsma, 2012). Optimization of soybean production and yields would therefore help narrow human food requirements and consequently help alleviate malnutrition in children and nutritional deficiencies in the elderly and people living with HIV and Aids.

For countries like Kenya, increased soybean production would help reduce huge importations of the crop and thus contribute to macroeconomic stability of the country. Apart from contributing to foreign exchange earnings through direct exports of the crop, soybean would also help provide raw materials to agro-based industries and in the process contribute to job creation in the country. Achievement of these benefits is however hampered by unavailability of information on how available soybean cultivars in Kenya respond to moisture stress. Understanding the response at physiological level is of significance considering that plant physiological processes have a direct bearing on crop yields (Liu et al*.,* 2012). Soil moisture stress interferes with key plant physiological processes like radiation use efficiency by photosynthesis, transpiration rate, level of stomata conductance, plant water status and degree of sub-stomatal carbon dioxide concentration in most crops (Ku et al., 2013; Hossain et al., 2014). It was for this reason that a study was conducted to determine the effect of varying soil moisture regimes on CO_2 assimilation of selected soybean cultivars in Kenya.

MATERIALS AND METHODS

Site description

The experiment was conducted in pots in a greenhouse at Egerton University, Njoro campus in Kenya, during 2017 and 2018 seasons. Egerton University (0° 22'S; 35°56'E) is at an altitude of 2267 meters above sea level (m.a.s.l) and had mean annual temperature of 15.9^{0}C.

Determination of moisture at field capacity

A sample of ten planting pots (18 cm in height and 22 cm in diameter giving a pot volume of 6,842 cm^3) used in the experiment were filled with soil and then saturated for several hours with water until all micro pores were filled with water. The top of the pots were then covered with black plastic sheets overnight to avoid evaporation. Moisture content at 100% field capacity (FC) was determined using IMKO-HD2 Time Domain Reflectometer (TDR) by inserting TDR probes vertically in the pot soil. The amount of moisture held by the soil at subsequent field capacities were then determined with reference to mean soil moisture level at 100% FC which was then used to come up with the following: 80, 60, 40, and 20% of FC. After sowing, moisture levels in all treatments were maintained close to 100% field capacity for 30 days after which respective soil moisture treatment regimes were initiated up to physiological maturity of the crop. After initiation of moisture regime treatments, soil moisture regimes at respective field capacities were monitored using TDR, and changes in soil moisture were corrected by supplying additional water.

Experimental design and treatments

The experiment was conducted using the Randomized Complete Block Design (RCBD) with a 4 x 6 factorial treatment arrangement with 3 replicates. Treatments consisted of two factors: factor 1 being moisture regimes and factor 2 being soybean cultivars. Soil moisture regimes were at 80%, 60%, 40% and 20% of soil moisture content at field capacity. Soybean cultivars used in the experiment were Gazelle, Nyala, EAI 3600, DPSB 8, Hill and DPSB 19. Characteristics of soybean cultivars are as follows (Table 1).

Planting and crop management

Soil growth medium was a mixture of clay loam soil and river sand in a 2:1 ratio. The growth medium was put in planting pots measuring 18 cm in height and 22 cm in diameter giving a pot volume of 6,842 cm^3. Planting pots were placed on a bench, at 100 cm above greenhouse floor. Natural lighting was used for plant growth and daily minimum and maximum temperatures were taken using a minimum and maximum bulb thermometer. Soybean seeds were inoculated with BIOFIX *(Bradyrhizobium japonicum)* inoculant strain USD 110 from Mea Limited–Kenya at the rate of 10 g kg^{-1} of seed prior to sowing. Three soybean seeds were sown in each pot and thinned to one plant per pot 14 days after emergence. Each treatment had 4 plants per replicate. Triple Super Phosphate (TSP) and Muriate of Potash (MOP) were applied as basal dressing fertilizers at the rates of 0.68 g per pot TSP (30 kg P_2O_5 ha^{-1}) and 0.27 g per pot (30 kg K_2O ha^{-1}), respectively. Hand weeding was done in pots as weeds appeared.

Determination of leaf relative water content

Leaf relative water content (LRWC) was measured on a third leaf from top of the plant at 50% flowering stage. Leaf samples were collected at midday and cut leaves were put in pre-weighed 150 milliliter tubes and sealed to avoid moisture loss. Closed tubes were put in an outdoor and indoor Marina cooler box and taken to laboratory where leaf fresh weights were measured. Equal amounts (150 milliliters) of distilled water were then added to tubes and samples placed in a refrigerator at 4^{o}C for 24 h for leaves to reach full turgor. After 24 h, leaf samples were removed from plastic containers, blotted dry with paper towel and weighed to get turgid weights. Leaf samples were then oven dried at 65^{o}C for 24 h after which dry weights were measured (Sade et al., 2015). Leaf relative water content was determined using the following formula:

LRWC (%) = [fresh leaf wt.-dry leaf wt. / leaf turgid wt.-dry leaf wt.]* 100%,

Where, LRWC is leaf relative water content.

Determination of leaf chlorophyll content

Chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll contents were

Table 1. Growth habits and phenology of soybean cultivars used in the experiment.

S/N	Cultivar name	Characteristics
1	Gazelle	Indeterminate, medium maturity
2	Nyala	Determinate, early maturity
3	EAI 3600	Determinate, early maturing
4	DPSB 8	Indeterminate, promiscuous, late maturity
5	Hill	Determinate, medium maturity
6	DPSB 19	Indeterminate, promiscuous, medium maturity

analyzed on a 3rd trifoliate leaf at 50% flowering using a procedure described by Goodwin and Britton (1988).

Measurement of stomata conductance

Stomata conductance was determined at 50% flowering and 50% podding stages of soybean growth on abaxial side of a middle leaflet of a third trifoliate leaf from top of the plant. It was measured between 12.00 - 14.00 hours on sunny days using a steady state leaf porometer (SC1, Decagon Devices, USA).

Measurement of leaf photosynthesis rate and sub-stomatal carbon dioxide concentration

Leaf photosynthesis rate and sub-stomatal CO_2 concentration were determined at 50% flowering and 50% podding stages of soybean growth on a middle leaflet of a 3rd trifoliate leaf from top of the plant. Photosynthesis rate and sub-stomatal CO_2 concentration were measured between 12.00 - 14.00 hours during sunny days using a TPS-2 portable photosynthesis system (V2.02-PP systems Inc., USA).

Statistical analysis

Data were checked for fulfilment of analysis of variance (ANOVA) assumption of normality by using Shapiro-Wilk normality test in Genstat release 18.1. Data that did not meet the aforesaid ANOVA assumption were subjected to a square root transformation before analysis. Data were then subjected to ANOVA using the linear mixed model for RCBD with factorial treatment arrangement in Genstat (Restricted Maximum Likelihood-REML) and statistically significant treatment means were separated using Tukey's test at 0.05 level of significance.

RESULTS

Leaf relative water content

Soil moisture regimes significantly influenced leaf relative water content (LRWC) in both 2017 and 2018 seasons (Figure 1). In 2017, moisture regimes at 80% FC and 60% FC registered LRWC which were significantly ($P < 0.001$) higher compared to LRWC registered at 40% FC and 20% FC. In 2018, 20% FC moisture regime significantly ($P< 0.01$) reduced LRWC while non-significant differences were observed amongst soil moisture regimes at 80% FC, 60% FC and 40% FC. While LRWC did not significantly differ amongst cultivars during 2017 season, LRWC significantly ($P \leq 0.05$) varied with cultivars during 2018 season (Figure 2). Cultivars DPSB 8 and Hill had highest and lowest LRWC during 2018 season, respectively.

Leaf chlorophyll content

Interactive effects of soil moisture regimes and cultivars on chlorophyll 'a' content was observed in both 2017 (Table 2) and 2018 seasons (Table 3). Soybean cultivars had highest ($P < 0.001$) chlorophyll 'a' content at lower soil moisture regimes of 40% FC and 20% FC during both seasons. While significant ($P < 0.001$) interactive effects of soil moisture regimes and cultivars for chlorophyll 'b' content was registered during 2017 season, soil moisture regimes, cultivars and their interactions were not significantly different for chlorophyll 'b' content during 2018 season. Overall, interaction of soil moisture regimes and soybean cultivars significantly ($P < 0.001$) influenced total chlorophyll concentration in soybean leaves in both seasons. Cultivar EAI 3600 had highest total chlorophyll content at the lowest soil moisture regime of 20% FC in both seasons.

Stomata conductance

Interaction of soil moisture regimes and cultivars significantly ($P < 0.001$) increased stomata conductance at 50% flowering and 50% podding stages of 2017 and 2018 seasons (Figures 3 to 6). All cultivars attained highest levels of stomata conductance at the least stressing moisture regime of 80% FC. During 2017 season, indeterminate cultivars DPSB 19 and DPSB 8 had highest stomata conductance at the most limiting soil moisture regime of 20% FC at 50% flowering and 50% podding stages, respectively. In 2018, highest stomata conductance levels at the lowest soil moisture regime were attained by cultivars DPSB 19 and EAI 3600 at 50% flowering stage and 50% podding stages, respectively.

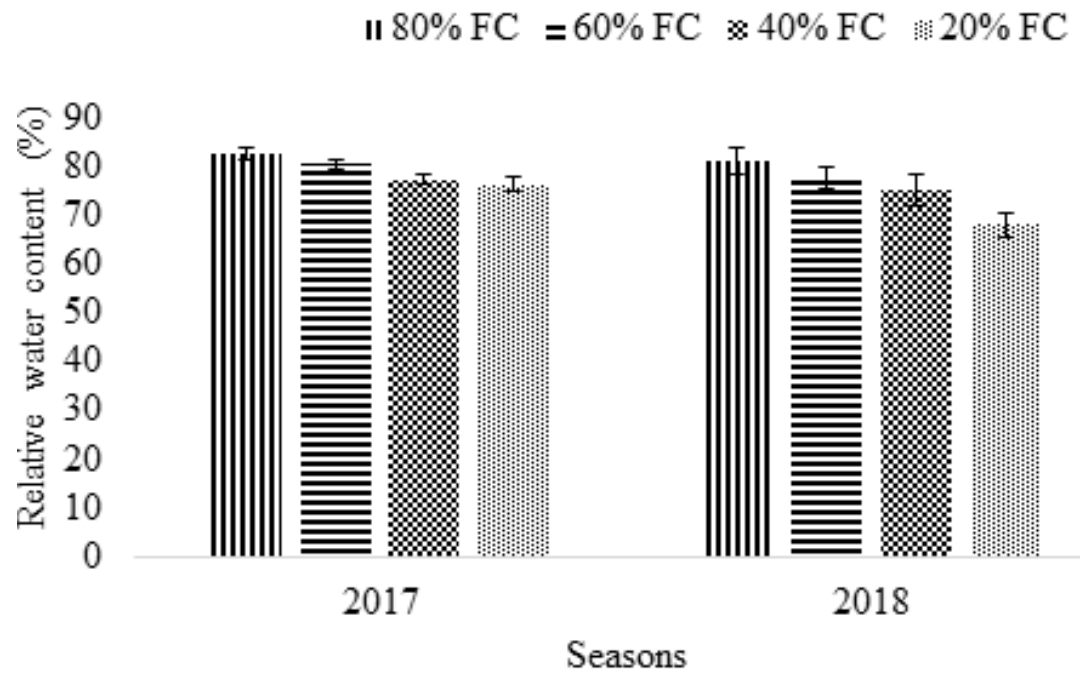

Figure 1. Effects of soil moisture regimes on leaf relative water content during 2017 and 2018 seasons (error bars represent standard error).

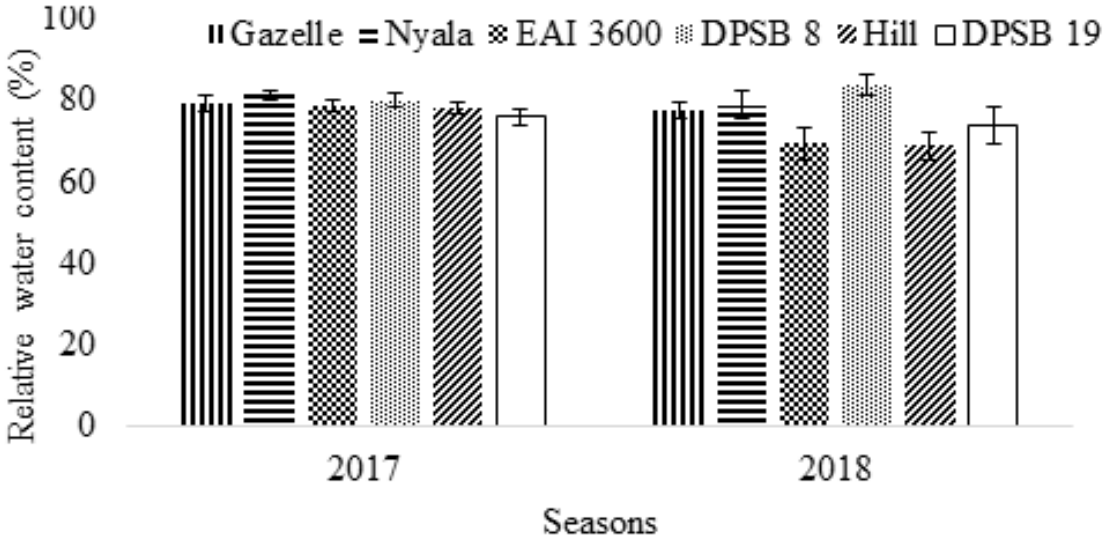

Figure 2. Effects of cultivars on leaf relative water content during 2017 and 2018 seasons (error bars represent standard error).

Sub- stomata CO_2 concentrations

Sub-stomatal CO_2 concentration at 50% flowering during 2017 season significantly ($P < 0.001$) varied with soil moisture regimes and cultivars. The highest sub-stomatal CO_2 concentration of 238.70 µmol CO_2 mol^{-1} was attained at the least limiting soil moisture level of 80% FC after which CO_2 concentrations progressively declined with increased soil moisture stress (Figure 7). Cultivar EAI 3600 had the highest sub-stomatal CO_2 concentration (242.42 µmol CO_2 mol^{-1}) though not statistically different from sub-stomatal CO_2 levels registered by cultivars

Table 2. Effects of soil moisture regimes and cultivars on soybean leaf chlorophyll content (mg g^{-1}fresh weight) at 50% flowering stage during 2017 season.

Soil moisture (% FC)	Cultivar	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll
		(mg g^{-1}fresh weight)		
80	Gazelle	0.97	0.12	1.09
	Nyala	0.92	0.12	1.04
	EAI 3600	1.03	0.12	1.15
	DPSB 8	0.86	0.11	0.99
	Hill	0.85	0.12	0.97
	DPSB 19	0.85	0.11	0.96
60	Gazelle	0.87	0.12	0.99
	Nyala	1.18	0.12	1.31
	EAI 3600	0.88	0.12	0.10
	DPSB 8	0.95	0.11	1.07
	Hill	0.85	0.13	0.96
	DPSB 19	0.89	0.11	1.01
40	Gazelle	0.87	0.12	0.99
	Nyala	0.93	0.12	1.06
	EAI 3600	0.87	0.11	0.98
	DPSB 8	0.92	0.13	1.05
	Hill	1.19	0.12	1.30
	DPSB 19	2.25	0.13	2.38
20	Gazelle	0.89	0.11	1.00
	Nyala	0.88	0.11	0.99
	EAI 3600	3.79	0.03	3.82
	DPSB 8	1.68	0.10	1.79
	Hill	1.04	0.13	1.16
	DPSB 19	0.87	0.10	0.96
P-value		<0.001	0.004	<0.001
$LSD_{(0.05)}$		0.515	0.03	0.513

FC = Field Capacity; LSD = Least significant Difference.

Gazelle (178.83 µmol CO_2 mol^{-1}) and DPSB 19 (148.11 µmol CO_2 mol^{-1}). While higher soil moisture levels significantly ($P \leq 0.05$) increased sub-stomatal CO_2 concentrations at flowering stage of 2018 season (Figure 8), soybean cultivars did not have significant influence. At 50% podding stage of both seasons, soil moisture regimes significantly ($P < 0.01$) increased sub-stomatal CO_2 concentrations with the highest and lowest levels attained at 80% FC and 20% FC respectively. Type of cultivar used did not yield any significant effects.

Photosynthetic rate

Photosynthetic rate was significantly ($P \leq 0.05$) increased with reduced soil moisture stress at 50% flowering stage of both seasons. In both cases, 80% FC had highest photosynthetic rate representing 64.46% (2017) and 63.27% (2018) increase over the lowest photosynthetic rates attained at 20% FC (Figures 9 and 10). Use of different soybean cultivars did not significantly influence photosynthesis at 50% flowering stage in both seasons. At 50% podding stage, both soil moisture regimes and cultivars did not give a significant effect on the rate at which photosynthesis was taking place.

Correlations between sub-stomatal carbon dioxide concentration and photosynthesis rate

Sub-stomatal carbon dioxide concentration andphotosynthesis rate of soybean cultivars showed a positive relationship (Figures 11 and 12). A linear relationship between carbon dioxide concentration and photosynthesis at 50% flowering stage indicates that the higher the concentration of sub-stomatal carbon dioxide, the greater the photosynthesis rate. Coefficient of determination (r^2) indicates that 87.75% and 93.42% of variations in photosynthesis rates at different soil moisture regimes in 2017 and 2018, respectively may be

Table 3. Effects of soil moisture regimes and cultivars on soybean leaf chlorophyll content (mg g^{-1}fresh weight) at 50% flowering stage during 2018 season.

Soil moisture (% FC)	Cultivar	Chlorophyll 'a'	Chlorophyll 'b'	Total Chlorophyll
		(mg g^{-1}fresh weight)		
80	Gazelle	0.82	0.10	0.92
	Nyala	0.38	0.06	0.45
	EAI 3600	0.84	0.10	0.94
	DPSB 8	0.58	0.12	0.70
	Hill	0.30	0.06	0.35
	DPSB 19	0.58	0.07	0.66
60	Gazelle	0.88	0.11	0.99
	Nyala	1.24	0.12	1.36
	EAI 3600	0.49	0.09	0.58
	DPSB 8	0.64	0.07	0.71
	Hill	0.53	0.09	0.61
	DPSB 19	0.53	0.07	0.61
40	Gazelle	0.42	0.62	0.48
	Nyala	0.49	0.07	0.56
	EAI 3600	0.73	0.10	0.83
	DPSB 8	0.89	0.08	0.98
	Hill	1.51	0.11	1.62
	DPSB 19	1.98	0.15	2.13
20	Gazelle	0.58	0.72	0.65
	Nyala	0.65	0.07	0.72
	EAI 3600	2.77	0.08	2.85
	DPSB 8	2.21	0.07	2.29
	Hill	0.54	0.06	0.60
	DPSB 19	0.65	0.08	0.72
P-value		<0.001	0.650	<0.001
$LSD_{(0.05)}$		1.186	0.090	1.228

FC = Field Capacity; LSD = Least significant Difference.

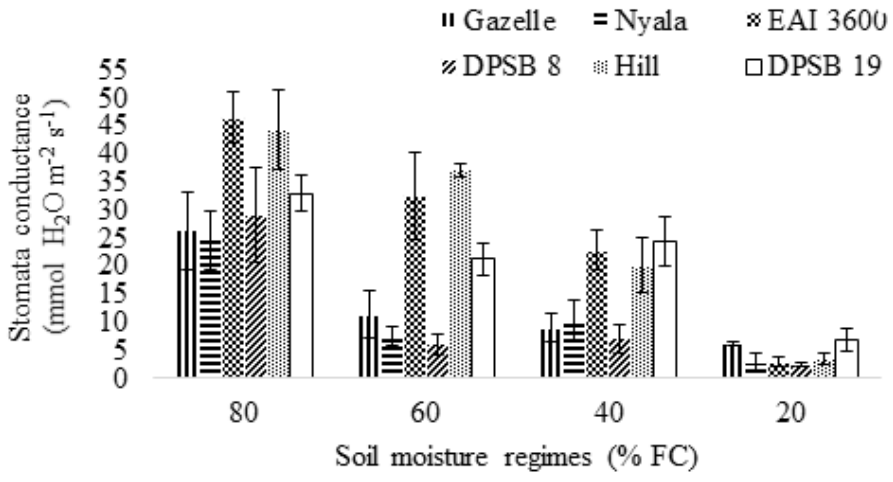

Figure 3. Effects of soil moisture regimes and cultivars on soybean stomata conductance at 50% flowering stage during 2017 season (error bars represent standard error).

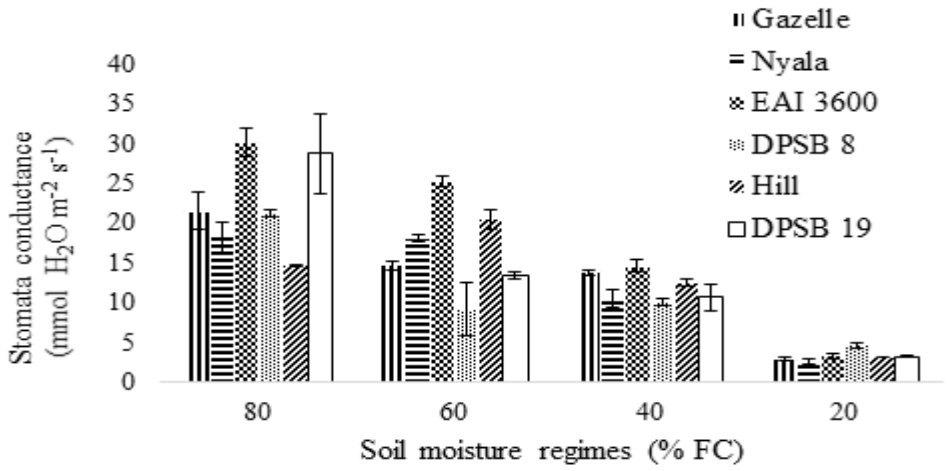

Figure 4. Effects of soil moisture regimes and cultivars on soybean stomata conductance at 50% podding stage during 2017 season (error bar represent standard error).

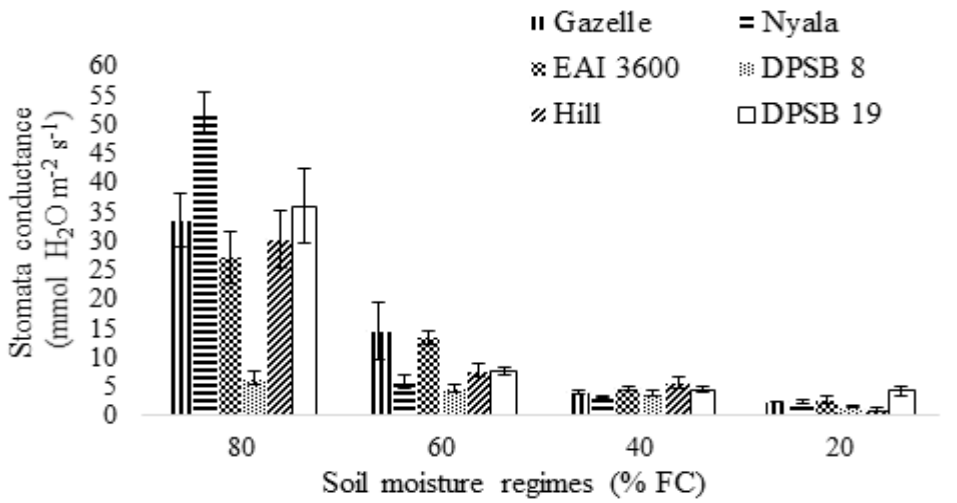

Figure 5. Effects of soil moisture regimes and cultivars on soybean stomata conductance at 50% flowering stage during 2018 season (error bars represent standard error).

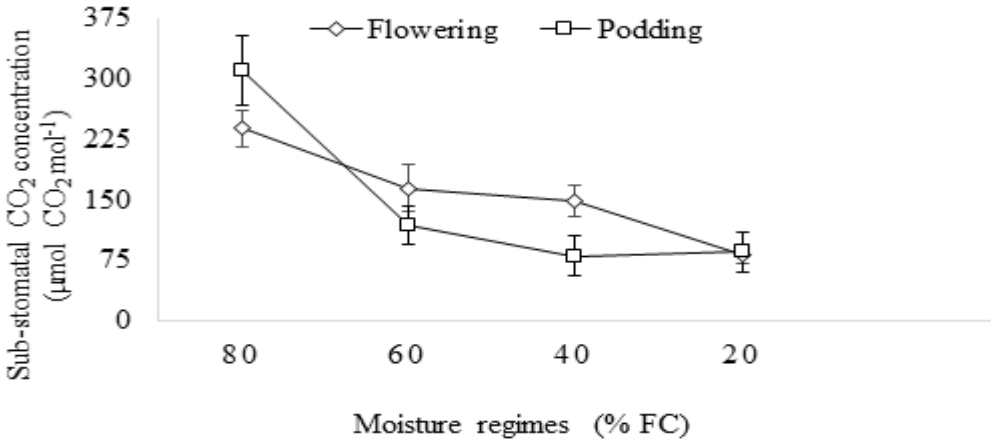

Figure 7. Response of sub-stomatal CO_2 concentrations to soil moisture regimes during 2017 season (error bars represent standard error).

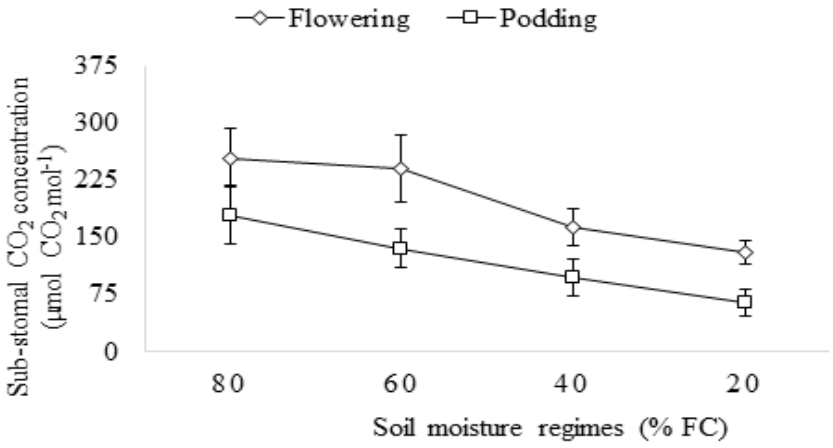

Figure 8. Response of sub-stomatal CO_2 concentrations to soil moisture regimes during 2018 season (error bars represent standard error).

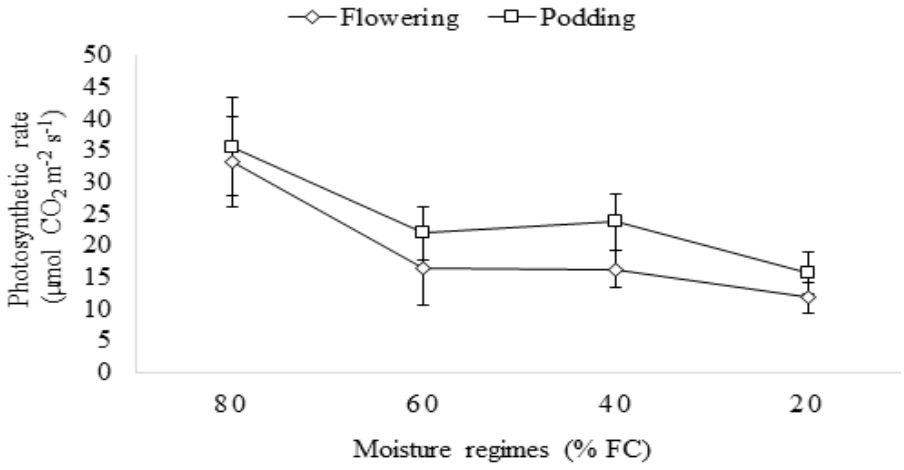

Figure 9. Effects of soil moisture regimes on soybean photosynthetic rate during 2017 season (error bars represent standard error).

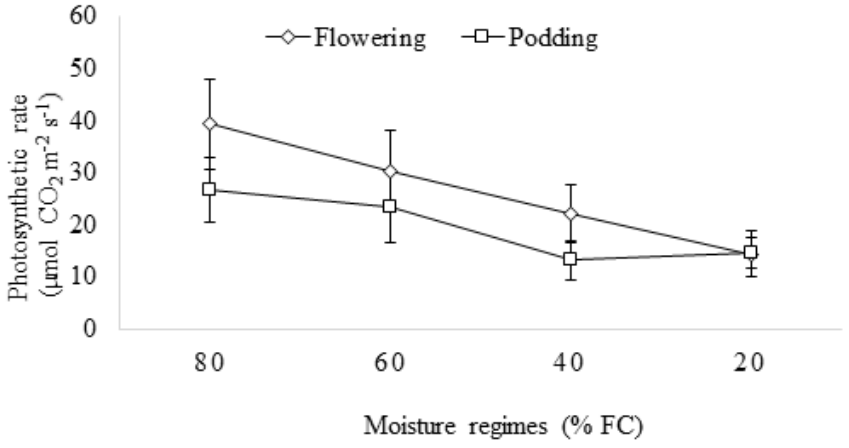

Figure 10. Effects of soil moisture regimes on soybean photosynthetic rate during 2018 season (error bars represent standard error).

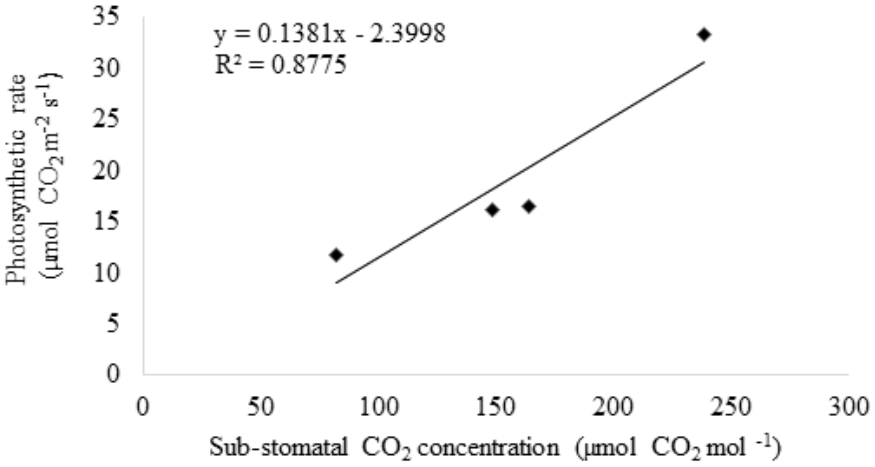

Figure 11. Correlation between sub-stomatal CO_2 concentration and photosynthetic rate at 50% flowering stage during 2017 season.

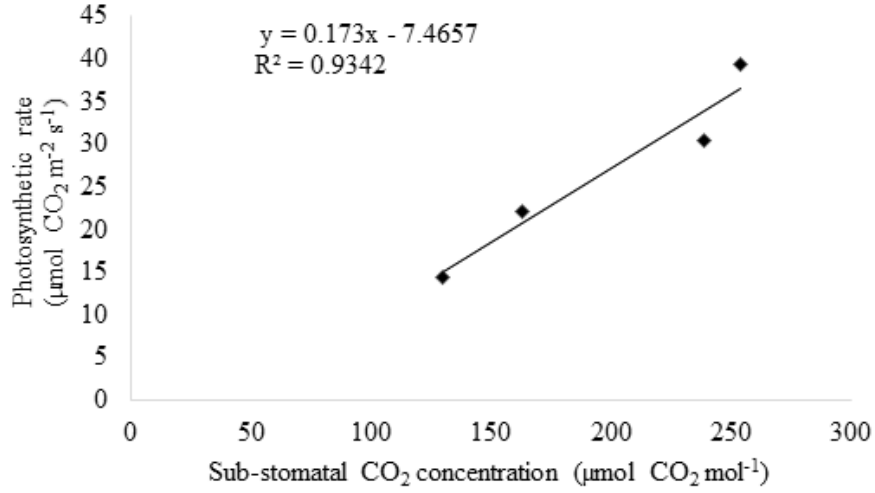

Figure 12. Correlation between sub-stomatal CO_2 concentrations and photosynthetic rate at 50% flowering stage during 2018 season.

attributed to differences in sub-stomatal carbon dioxide concentrations.

DISCUSSION

Leaf relative water content measures the dehydration status of plants relative to the maximum water holding capacity at full turgidity. A cultivar with the ability to minimize stress by maintaining turgid leaves under limited soil moisture conditions may be considered drought tolerant (Lugojan and Ciulca, 2011; Soltys-Kalina et al., 2016). Results of the study have shown that soil moisture stress reduced leaf relative water content with cultivars DPSB 8, Nyala, Gazelle and DPSB 19 maintaining higher percent leaf relative water content which signifies moisture stress tolerance potential of the cultivars. Previous studies on soybean have also demonstrated that soil moisture stress reduces leaf relative water content with a pronounced effect on moisture stress susceptible cultivars (Amira and Qados, 2014; Hossain et al., 2014). Under limited soil moisture conditions, there is lower cell water potential which may lead to reduced leaf relative water content in plants grown under such conditions (Cheruiyot et al., 2010., Hossain et al., 2015). In drought tolerant soybean cultivars, high leaf relative water content is maintained by the increased expression of P5CS gene resulting in increased biosynthesis of proline, which helps in cell stabilization and maintenance of cell turgidity (Hayat et

al., 2012).

Chlorophyll ‘a’ is the principal photosynthesis pigment that interacts directly with light requiring processes of photosynthesis. Chlorophyll ‘b’, on the other hand, is an accessory photosynthesis pigment and it acts indirectly in photosynthesis process by transferring light it absorbs to chlorophyll ‘a’. A combination of chlorophylls ‘a’ and ‘b’ constitutes total chlorophyll content in plant leaves (Guidi et al., 2017). This study has shown that chlorophyll ‘a’ and total chlorophyll content of soybean leaves increased with increased soil moisture stress in both seasons. There was no explicit effect of soil moisture regimes on chlorophyll ‘b’ concentration considering that during 2017 season, chlorophyll ‘b’ content was significantly increased at higher soil moisture regimes while soil moisture regimes did not have a notable significant influence, despite a trend of higher chlorophyll ‘b’ content at higher soil moisture regimes in 2018. In 2017 season, chlorophylls ‘a’ and ‘b’ including total chlorophyll concentration varied with soybean cultivars used. In 2018, however, all chlorophyll components were not significantly influenced by soybean cultivars. Contradicting results on effect of soil moisture stress on leaf chlorophyll content have been reported from previous studies. Significant decreases in chlorophyll ‘a’, ‘b’ and total chlorophyll content in leaves of soybean plants grown under drought stress were reported by Atti et al. (2014) and Mannan et al. (2016). Nonetheless, a studies on corn by Rahman et al. (2004) and Muhumed et al. (2014) indicated an increase in total chlorophyll content with increase in water stress, with corn cultivars showing an inverse relationship in increases of chlorophylls ‘a’ and ‘b’. Maintaining high soil moisture regimes in this study required frequent application of water which might have led to leaching of nutrients from growth medium. This might have deprived soybean plants of the required nitrogen to sustain high chlorophyll levels. Reduced nitrogen contents in sweet corn leaves and roots as a result of increased irrigation frequencies were reported by Muhumed et al. (2014).

Highest levels of stomata conductance, sub-stomatal CO_2 concentration and photosynthesis rates were attained at highest soil moisture regime of 80% FC, with largely limited variations amongst plant growth stages and cultivars. Higher photosynthesis rate was highly correlated with higher concentration of sub-stomatal CO_2. These results are in agreement with observations by Makbul et al. (2011), Hossain et al. (2015) and Chowdhury et al. (2016) who reported reductions in stomata conductance, sub-stomatal CO_2 concentration and photosynthesis rate due to increased moisture stress in soybean plants grown under greenhouse conditions and other related growth chambers. Catuchi et al. (2011) and Fanourakis et al. (2014) indicated that most plants close stomata at limited soil moisture levels to prevent excess water loss to the environment. Closure of stomata by plants at limited soil moisture levels in the current study triggered a series of events in plant physiological processes. Reduced stomata conductance at lower soil moisture regimes might have arisen from a combination of reductions in relative water content in soybean leaves and stomata closure to prevent excess water loss to the environment. Considering that stomata conductance indicates a degree of exchange of CO_2 and water vapour between ambient and inner leaf, reduced stomata conductance due to stomata closure could have then led to minimal diffusion of CO_2 from the atmosphere to plant cells leading to low concentrations of sub-stomatal carbon dioxide (Fanourakis et al., 2014). It has been shown from this study that photosynthesis rate was strongly correlated with sub-stomatal CO_2 concentrations which implies that lower photosynthesis rate at lower soil moisture regimes could have been a result of reduced sub-stomatal CO_2 diffusion to carboxylation site of Rubisco (Xu et al., 2016).

Conclusion

Soil moisture stress reduced leaf relative water content, stomata conductance, sub-stomatal carbon dioxide concentration and photosynthesis rate, while leaf chlorophyll content increased with increased soil moisture limitation. Cultivars DPSB 19 and DPSB 8 had relatively higher leaf relative water content and stomata conductance at reduced soil moisture regime of 20%, indicating moisture stress tolerance potential of the cultivars.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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Full Length Research Paper

Physiological quality of colza seeds (*Brassica napus* L.) after coating and seed treatment during storage

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Seed coating is a technique widely used by growers to increase, standardize and improve seed germination conditions. Despite this, there is lack of information about the physiological quality of coated seeds during storage. The objective of this study is to evaluate the physical and physiological quality of colza (*Brassica napus* L.) seeds coated with bentonite and treated with fungicide and black pepper plant extract within 120 days of storage. Colza seeds were coated with bentonite as a filler material and polyvinyl acetate (PVA) glue as cementing material. For treatment of the seeds a fungicide (Carboxin + Thiram) and black pepper (*Piper nigrum* L.) plant extract were added together with the cementing material. Seeds with no coating or treatment were used as controls. After coating, the seeds were stored for 120 days, and the first germination counting, germination, third counting, shoot dry matter and water content were evaluated every 30 days. Germination and vigor of canola seeds coated and treated with fungicide or black pepper plant extract decreased significantly throughout the storage period. At 120 days of storage, the germination of the seeds coated with bentonite + glue and bentonite + plant extract was at an average of 33.6% in the third counting.

Key words: *Brassica napus* L, water content, seed coating, bentonite.

INTRODUCTION

Colza seeds (*Brassica napus* L.) are the third most-produced oilseeds in the world. It is an annual herbaceous plant belonging to the Brassicaceae family, which produces high quality oil-rich grains. This culture is responsible for 15% of the world's edible vegetable oil production, although it is also used in the production of biodiesel and animal feed (Tomm, 2007).

The use of machines in agriculture is of known importance. They are responsible for the expansion of areas of cultivation and productivity. According to Beltrão

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and Vieira (2001) one of the obstacles in mechanized sowing is the seed with small size, irregular shape and light. Colza seeds, according to Angelotti-Mendonça et al. (2016) have such characteristics. Seeds no longer represent only a propagule of a crop; they also carry a new way of managing agricultural technology. The value added to the seeds, methods and technologies of production of seed coating are the main requirements of an increasingly competitive market. For this, seeds with high germination/emergence uniformity and production of seedlings with high growth potential are required (Baudet and Peres, 2004).

According to Lopes and Nascimento (2012) coating/pelleting is a technology that is based on the sedimentation of dry inert materials with fine granulometry on the surface of the seeds with the aid of a cementing material. According to Nascimento et al. (2009), this technology allows the uniformization of the form, increase the size and weight of the seeds. Thus, sowing is facilitated, when it is done manually or through machines (Nascimento et al., 2009). Another important practice used during the coating process is the application of chemical products for the treatment of seeds, aiming the control and/or protection against insects, microorganisms and rodents (Sampaio and Sampaio, 1994). The process consists basically of applying successive layers of an inert solid material to the seeds in constant movement within a concrete mixer, alternating the application of the filler material with the spraying of a water-soluble binder (Silva, 1997; Silva and Nakagawa, 1998).

Although the technique has been developed for several years, information on the composition of the materials used and the preparation of the coatings are not well known, since this technique remains inaccessible to the seed and seed conditioning companies (Silva et al., 2002). Even with potential for utilization, there is lack of information available on seed coatings, encrustation or pelleting, especially regarding coating composition and seed performance during storage (Oliveira et al., 2003).

Based on that, the study of materials used for the seed coating, in association or not with products for treatment, whether chemical or natural, is of fundamental importance, supplying information in this area of research, especially when dealing with the storage of coated seeds. The objective of this study is to evaluate the physical and physiological quality of colza (*B. napus* L.) seeds during storage, coated with bentonite and treated with fungicide or plant extract.

MATERIALS AND METHODS

Locale of the experiment

The experiment was carried out at the Laboratório de Armazenamento e Processamento de Produtos Agrícolas, of the Universidade Federal de Campina Grande, Campus of Campina Grande, Paraíba, Brazil.

Plant extract preparation

Fruits of Black Pepper (*Piper nigrum* L.) were purchased at the central fair of the city of Campina Grande, Paraíba, Brazil. The aqueous extract was obtained from the fruits powder, which were weighed, moistened with distilled water, and placed for maceration for 72 h, at room temperature of 24.0 ± 4.0°C, in the absence of light and with daily agitation for five minutes. The amount of powder used corresponded to 20% of the volume of water used. The final solution was filtered on filter paper; the extract was stored in an amber glass container with of 0.5 L volume (Almeida et al., 2004).

Seed coating and storage of the seeds

The colza seeds (*B. napus* L.) were submitted to the coating process that occurred by alternating application between cementing material and filler material. This process was repeated until a complete utilization of the materials. These seeds were coated with bentonite and treated with fungicide (Carboxin + Thiram) and aqueous black pepper plant extract *(P. nigrum* L.) (the volume of vegetal extract and fungicide corresponded to 10% of the volume of cementing material). After 24 h of coating, the seeds were stored under ambient conditions of temperature (24.02 ± 5.85°C) and relative humidity (82.08 ± 5.85%) in acrylic containers (thickness 1.5 mm), with capacity for 2000 seeds (50 ml), for 120 days. The germination tests and vigor were performed every 30 days. Seeds with no coating or treatment were used as controls.

Germination test

The germination test was performed with four sub-samples of 50 seeds, sowing them in plastic trays with vermiculite, moistened with distilled water corresponding to 60% of the retention capacity of the substrate. These were maintained under ambient conditions of temperature, relative humidity and photoperiod. Germination was evaluated on the seventh day after the start of the test and the first counting was performed on the fourth day after sowing (Brasil, 2009). A third germination counting was also performed at 14 days after sowing to evaluate whether there was a decrease or delay of germination.

Shoot dry matter

To determine the shoot dry matter, the seedlings were cut at the substrate level and placed in *kraft* paper bags. The bags were placed in a lab stove with forced air circulation for drying, at a temperature of 65 °C until a constant weight was reached. After that, the plant material was weighed in a precision digital scale and the data expressed in milligrams (mg).

Water content

The water content was determined by the standard lab stove method at 105 ± 2°C, where four sub-samples of 2.0 g of seeds were placed in metal containers previously dried in a greenhouse and weighed. After 24 h the containers with the samples were placed in a desiccator for 20 min, until they reached room temperature and weighed, obtaining the final weight (containers plus the dry sample). The results were expressed as percentage of weight in wet basis according to the equation contained in the Rules for Seed Analysis (Brasil, 2009).

Experimental design and statistical analysis

The experiment was arranged in a completely randomized design and arranged in a 4 x 5 factorial scheme (coating materials x

storage periods); each treatment had four replications. The data were submitted to Analysis of Variance (P ≤ 0.05). For the quantitative factor (storage periods); the data were submitted to regression in the Variance Analysis to determine the models for each material. For the qualitative factor (coating materials) the means, when necessary, were compared by the Scott-Knott test (P ≤ 0.05). In addition, the germination data were corrected, considering the control in time 0 days as 100%. For all statistical analyses the software Assistat, version 7.6, was used.

RESULTS

Table 1 shows the mean squares values for water content (WC), first germination counting (FGC), germination (G), third germination counting (TGC) and shoot dry matter (SDM). The effect of interaction between the factors (coating materials × storage periods), except for FGC, was observed for all variables under study, where a highly significant effect was observed for the isolated factors. The observed interactive effects reveal statistical differences between factor treatments. When comparing the coating materials within each storage period, it was observed that for 0 and 30 days of storage the highest water content was verified, when the seeds were coated with bentonite + fungicide. On the other hand, the lowest water contents, for these same periods of storage, were verified in the control. When the seeds were coated with bentonite + glue and bentonite + plant extract, the water contents were intermediate to the other materials (Table 2).

At 60, 90 and 120 days of storage it is observed that there was no statistical difference between the coating materials for water content. However, the water content has statistically different values when the variants with coating materials and the control are compared (Table 2).

Figure 1 shows the water content of seeds coated with bentonite and treated with fungicide or black pepper extract during 120 days of storage. According to the regression analysis of variance, for bentonite + glue and bentonite + fungicide the analysis did not reveal any significant model. For the other combinations, the only significant models were of second degree; however, they presented values of R^2 very low (0.19 and 0.50), being chosen graphically to present the behavior of the data and the means. In general, no significant variations were observed in the water content of the coated seeds or not during storage.

No statistical difference was observed for the coating materials within each storage period for the first germination counting. However, a statistical difference was found between the means of the factor "coating materials", regardless of storage periods. There was a higher germination for the control, differing statistically from the germination of seeds that were coated with bentonite + PVA glue and bentonite + plant extract. The seeds coated with bentonite + fungicide had the lowest germination compared to the others treatments studied, differing statistically from the other coating materials and the control (Table 3). When comparing the coating materials within each storage period, it was observed that for the periods 0, 30, 60 and 90 days, the lowest germinations were verified for the seeds coated with bentonite + fungicide. On the other hand, the highest germinations, for these same periods of storage, were verified in the control. The germinations observed in seeds coated with bentonite + PVA glue and bentonite + plant extract, presented an intermediate behavior, differing statistically among them, in all storage periods mentioned above. At 120 days of storage, the highest germination was verified in the control, differing statistically from the germination of seeds coated with bentonite + PVA glue and bentonite + plant extract, which were statistically similar to each other, and different from the germination of seeds coated with with bentonite + fungicide, with no germination (Table 4). The models of highest degree that best fit the germination data of coated seeds or not according to storage periods were first degree for bentonite + fungicide and bentonite + plant extract, being significant at 1%, and of second degree for bentonite + PVA glue, significant at 5%. For the control, the analysis revealed no significant model. The models presented coefficients of determination ranging from 0.9275 to 0.9831, representing the experimental data satisfactorily.

It can be observed that in the control, the germination had low influence by the storage period. The germination of the seeds coated with bentonite + PVA glue practically remained until 60 days of storage, however, after this period there was a decrease of germination as the storage time increased. The seeds coated with bentonite + fungicide and bentonite + plant extract had their germination decreased after 30 days of storage, continuing until the end of the storage period (Figure 2).

The means of the third counting of germination of the seeds coated with bentonite and treated with fungicide or black pepper extract are presented in Table 5. Comparing the coating materials within each storage period it can be verified for the time 0 (zero) that the greatest germination was found in the control, differing statistically from the germination percentage observed in coated seeds, which did not differ statistically among them. At 30 days of storage, the highest germination was verified in the control. On the other hand, the lower germinations, in the third counting, were observed for the seeds coated with bentonite + fungicide and bentonite + plant extract. Seeds that were coated with bentonite + PVA glue exhibited intermediate performance, differing statistically from the other coating materials and the control (Table 5). At the periods of 60 and 90 days of storage it could be verified that all the coating materials were statistically different from each other. The lowest germinations were observed in seeds coated with bentonite + fungicide. Differently, the highest germinations were observed in the control. The other treatments had intermediate values, differing statistically

Table 1. Mean squares for water content (WC), first germination counting (FGC), germination (G), third germination counting (TGC) and shoot dry matter (SDM) of colza seedlings coated with different filler materials (FM) during the storage period (SP).

SV	DF	Average square				
		WC	FGC	G	TGC	SDM
FM	3	32.339**	9021.916**	12352.983**	7708.850**	49741.666**
SP	4	0.007*	504.325**	31.782**	2010.550**	42.861**
FM x SP	12	0.005**	1.568ns	3.059**	300.683**	3.729**
Error	60					

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

Table 2. Water content (%) of colza seeds coated with bentonite and treated with fungicide and black pepper extract during 120 days of storage.

Coating material	Storage period (days)				
	0	30	60	90	120
T	5.07±0.10^{c}	5.12±0.07^{c}	5.06±0.08^{b}	5.03±0.16^{b}	5.07±0.07^{b}
B + G	7.40±0.03^{b}	7.44±0.07^{b}	7.48±0.12^{a}	7.44±0.04^{a}	7.45±0.24^{a}
B + F	7.84±0.19^{a}	7.91±0.16^{a}	7.86±0.17^{a}	7.83±0.12^{a}	7.78±0.12^{a}
B + PE	7.43±0.17^{b}	7.46±0.12^{b}	7.50±0.11^{a}	7.44±0.09^{a}	7.53±0.13^{a}

* Means followed by the same lowercase letter in the column do not differ by Scott-Knott's test (P ≤ 0.05). CV% = 4.25. (T) control; (B + G) bentonite + PVA glue; (B + F) bentonite + fungicide; (B + PE) bentonite + plant extract.

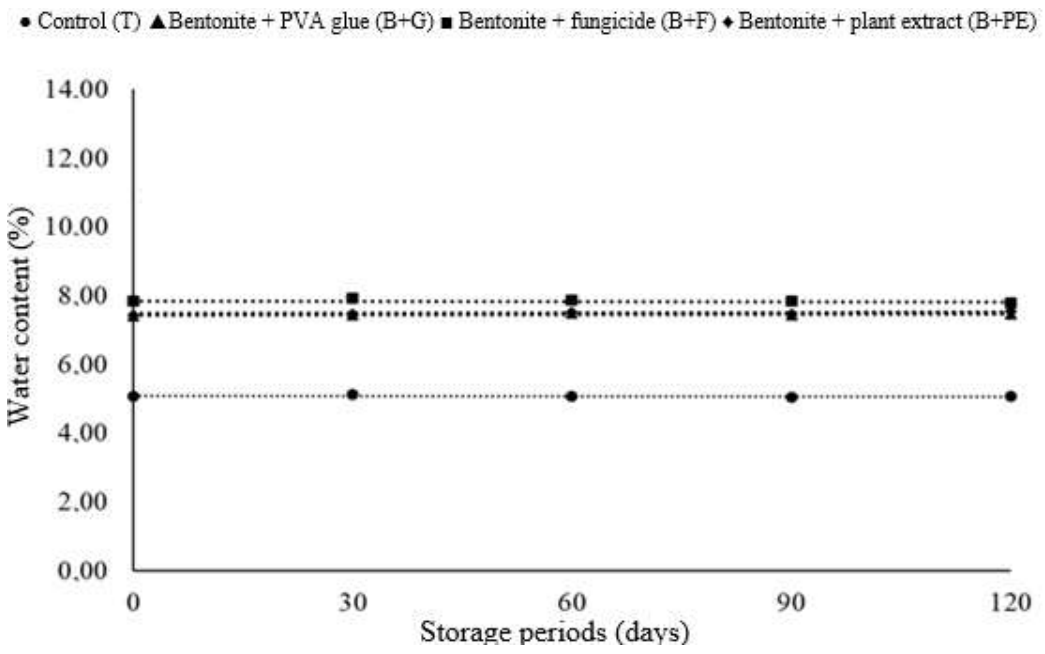

Figure 1. Water content of colza seeds coated with bentonite and treated with fungicide and black pepper extract during 120 days of storage, compared to control.

from each other and the other treatments (Table 5). At 120 days of storage, the highest germination occurred in the control, differing statistically from the germinations observed in seeds coated with bentonite + PVA glue and bentonite + plant extract, which were statistically equal to each other and different from the germination verified of seeds coated with bentonite + fungicide, which was the coating material that provided lowest germination among

Table 3. First germination counting (%) of colza seeds coated with bentonite and treated with fungicide and black pepper extract during 120 days storage.

Filler material	Storage period (days)					Average
	0	30	60	90	120	
T	57.5 ± 0.83 -100	58.0 ± 1.22 -100	57.0 ± 0.50 -99.1	55.0 ± 2.05 -95.7	46.5 ± 0.83 -80.9	54.80[a] -95.3
B + G	34.5 ± 2.49 (60.0)	30.5 ± 1.64 -53	26.0 ± 1.58 -45.2	18.0 ± 1.87 -31.3	16.0 ± 2.74 -27.8	25.00b -43.5
B + F	8.0 ± 1.58 -13.9	8.0 ± 2.83 -13.9	6.0 ± 2.35 -10.4	1.0 ± 0.50 -1.7	0.0 ± 0.00 0	4.60[d] -8
B + PE	26.0 ± 2.45 -45.2	23.0 ± 1.12 -40	18.0 ± 2.83 -31.3	12.5 ± 1.64 -21.7	10.0 ± 0.71 -17.4	17.90[c] -31.1
Average	31.5 -55	29.88 -52	26.75 -47	21.63 -37.6	18.13 -31.5	

*Means followed by the same lowercase letter in the column do not differ by Scott-Knott's test (P ≤ 0.05). CV% = 16.21 (C) control; (B + G) bentonite + PVA glue; (B + F) bentonite + fungicide; (B + PE) bentonite + plant extract. *Means in parentheses corrected to 100%.

Table 4. Germination (%) of colza seeds coated with bentonite and treated with fungicide and black pepper extract during 120 days of storage.

Filler material	Storage period (days)				
	0	30	60	90	120
T	71.5±1.92[a] -100	71.5±1.92[a] -100	71.0±2.50[a] -93.9	68.5±2.68[a] -95.8	67.0±2.69[a] -93.7
B + G	54.0±2.12[b] -75.5	50.5±2.38[b] -70.6	49.0±3.50[b] -68.5	41.0±4.03[b] -57.3	24.0±4.95[b] -33.6
B + F	23.0±2.50[d] -32.2	15.5±1.09[d] -21.7	13.0±1.50[d] -18.2	6.5±3.49[d] -9.1	0.0±0.00[c] 0
B + PE	44.0±1.22[c] -61.5	30.0±1.87[c] -42	29.0±2.69[c] -40.6	19.5±0.83[c] -27.3	16.0±1.58[b] -22.4

*Means followed by the same lowercase letter in the column do not differ by Scott-Knott's test (P ≤ 0.05). CV% = 15.17. (C) control; (B + G) bentonite + PVA glue; (B + F) bentonite + fungicide; (B + PE) bentonite + plant extract.

the materials (Table 5).

According to the regression in the analysis of variance, the highest degree model, that best fit the data of the third germination counting as a function of the storage periods, was the second degree, for all the coated seeds, being significant at 1% for bentonite + plant extract and 5% for bentonite + glue and bentonite + fungicide. The determination coefficients for these models ranged from 0.9658 to 0.9999, representing reliably the experimental data from the third germination counting (Figure 3). It was verified that in the control there was no significant decrease in the third counting of germination during the storage period. For the seeds coated with bentonite and PVA glue, the germination tended to decrease up to 60 days of storage, but slowly. After that, an accentuated decrease of germination was verified. For the seeds coated with bentonite + fungicide a decrease of the germination was verified at 30 days of storage, maintaining this behavior until the end of storage. It can be verified that the seeds coated with bentonite + plant

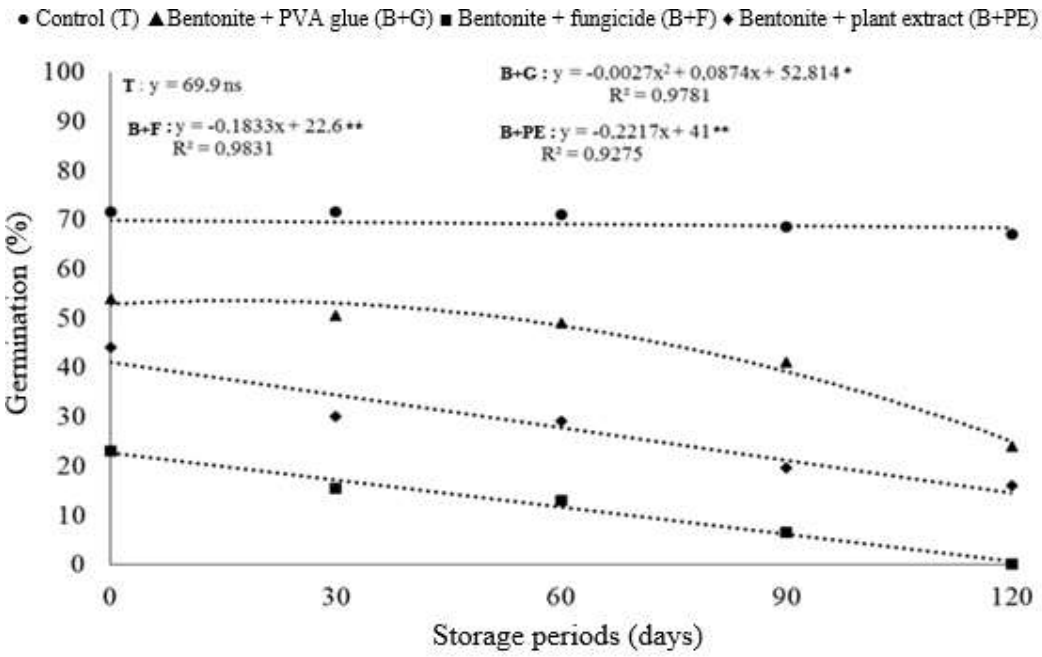

Figure 2. Germination of colza seed coated with bentonite and treated with fungicide and black pepper extract during 120 days of storage, compared to control.

Table 5. Third germination counting (%) of bentonite-coated colza seeds and treated with fungicide and black pepper extract during 120 days of storage.

Filler material	Storage periods (days)				
	0	30	60	90	120
T	72.0±0.71[a] -100	72.0±1.58[a] -100	71.5±2.17[a] -99.3	69.0±2.29[a] -95.8	67.0±2.69[a] -93.1
B + G	59.0±1.66[b] -81.9	54.0±1.00[b] -75	50.0±3.03[b] -69.4	43.5±3.96[b] -60.4	24.5±5.31[b] -34
B + F	55.5±1.92[b] -77.1	37.0±0.43[c] -51.4	22.5±1.09[d] -31.3	11.0±3.77[d] -15.3	4.0±1.22[c] -5.6
B + PE	49.5±0.43[b] -68.8	33.5±1.92[c] -46.5	30.0±3.03[c] -41.7	23.0±1.80[c] -31.9	24.0±1.09[b] -33.3

*Means followed by the same lowercase letter in the column do not differ by Scott-Knott's test (P ≤ 0.05). CV% = 12.68. (C) control; (B + G) bentonite + PVA glue; (B + F) bentonite + fungicide; (B + PE) bentonite + plant extract.

extract had their germination reduced at 30 days of storage which continued until up to 90 days of storage. After this period the germination in the third counting tended to remain (Figure 3).

Comparing the coating materials within each storage period, it could be verified that at the time 0 (zero), the highest value for dry matter was verified in the control, differing statistically from the dry matter of seedlings from seeds coated with bentonite + PVA glue and bentonite + plant extract, which were statistically equal to each other and different from bentonite + fungicide, which had the lowest value for dry matter (Table 6). For the other storage periods, it was verified that all the coating materials were statistically different, with the highest values for shoot dry matter being verified in the control. On the other hand, the lowest values for shoot dry matter were verified when the seeds were coated with bentonite + fungicide (Table 6). The regression of the analysis of

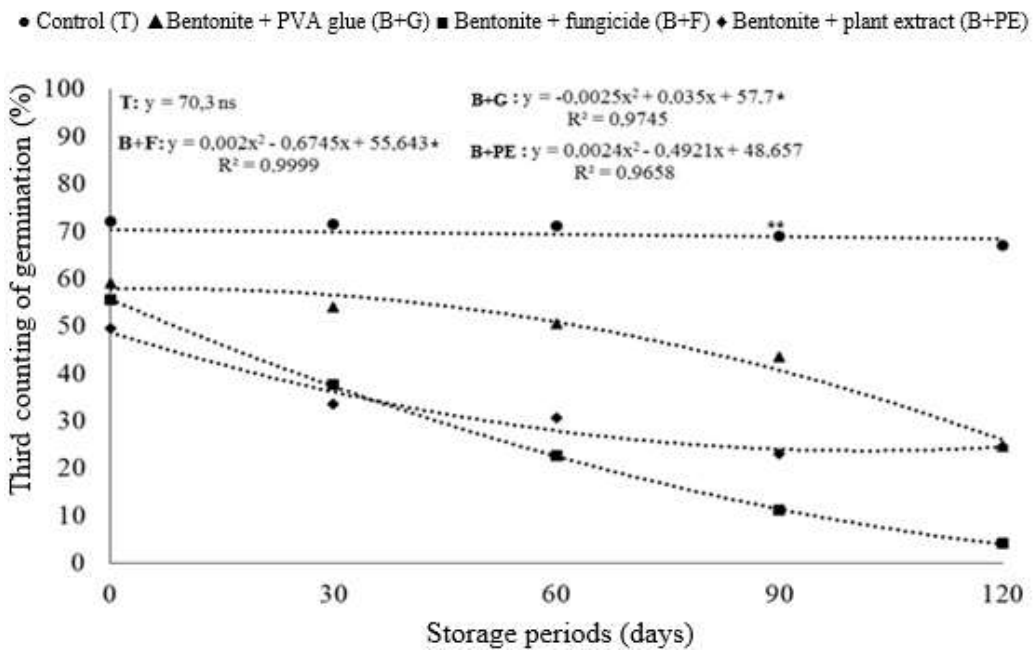

Figure 3. Third counting of germination of colza seeds coated with bentonite and treated with fungicide and black pepper extract during 120 days of storage.

Table 6. Shoot dry matter of seedlings (mg) of colzacoated with bentonite and treated with fungicide and black pepper extract during 120 days storage.

Filler material	Storage periods (days)				
	0	30	60	90	120
T	140.0±3.54[a]	137.5±2.17[a]	135.0±2.50[a]	127.5±4.15[a]	127.5±8.93[a]
B + G	92.5±2.17[b]	82.5±5.45[b]	80.0±7.07[b]	62.5±4.15[b]	35.0±5.59[b]
B + F	37.5±2.17[c]	22.5±5.45[d]	15.0±2.50[d]	10.0±3.54[d]	0.0±0.00[d]
B + PE	80.0±3.54[b]	52.5±4.15[c]	45.0±5.59[c]	27.5±2.17[c]	15.0±2.50[c]

*Means followed by the same lowercase letter in the column do not differ by Scott-Knott's test (P ≤ 0.05). CV% = 15.16. (C) control; (B + G) bentonite + PVA glue; (B + F) bentonite + fungicide; (B + PE) bentonite + plant extract.

variance reveals that for the control and for seeds coated with bentonite + PVA glue, the model of highest degree that best fit the experimental data of the shoot dry matter as a function of storage periods was the second degree, where the first treatment was significant at 1% and for the second treatment, significant at 5%, having determination coefficients of 0.9245 and 0.9797, respectively. For bentonite + fungicide and bentonite + plant extract the model of highest degree that best fit the experimental data was the first degree, with determination coefficients of 0.9661 and 0.9678, respectively. Regarding the determination coefficients, all of them reliably represent the experimental data, with R^2 above 92% (Figure 4). For all treatments, a decrease in shoot dry matter was observed during the storage period. For bentonite + PVA glue, a slight decrease was verified up to 60 days of storage, with a more accentuated decrease after this period (Figure 4). For the seeds coated with bentonite + fungicide and bentonite + plant extract, a decrease of shoot dry matter of the seedlings was observed as the time of storage increased (Figure 4).

DISCUSSION

It could be observed that the water content varied for bentonite at 0 and 30 days, having higher percentages when the bentonite was used together with the fungicide. For the other storage periods, no difference was observed between products of treatment with bentonite. It can be seen that all combinations of bentonite and treatment product had higher water contents than the

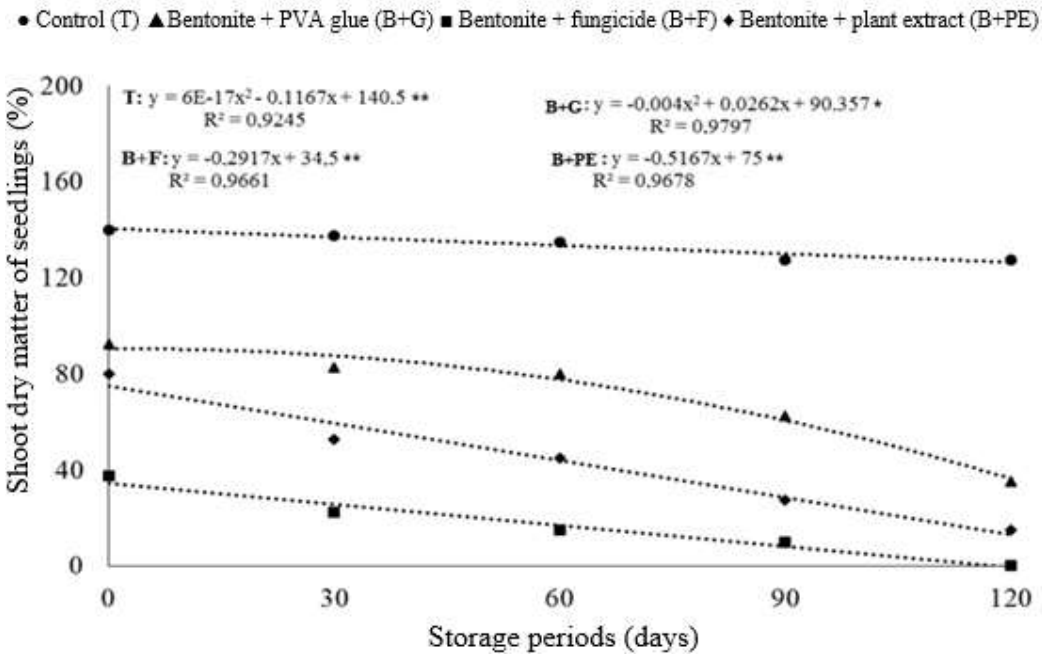

Figure 4. Shoot dry matter of seedlings of colza coated with bentonite and treated with fungicide and black pepper extract during 120 days of storage.

control. This fact is explained by the high swelling capacity of bentonite in the presence of water, since it is a material that can retain water (Silva and Ferreira, 2008). Another aspect observed is that the water contents of the seeds, coated or not, presented little variation during the storage. This can be explained by the fact that they were stored in acrylic containers and in laboratory conditions, where there is little variation of climatic conditions.

All parameters of physiological seed quality evaluated in this studied were negatively affected by the coatings during storage, especially those seeds coated with bentonite + fungicide. The fungicide caused an acceleration in the deterioration of the seeds during storage, observing total inhibition of germination at 120 days of storage. Differently, non-coated seeds exhibited a decrease in germination and vigor in a slight manner during storage. The non-coated seeds had an average germination of 70%, which accentuated the effect of the treatments. However, colza seed is a difficult material to obtain in our region, being the only material available at the time of installation of the experiment. Another aspect noticed, is that in the third counting of germination the seeds had a greater recovery of the germination in the time 0 (zero), differently from what was observed at 120 days of storage. In the second and third counting, at time 0 (zero), a decrease of approximately 20% occurred when compared with the control. On the other hand, at 120 days of storage this decrease was approximately 60% when compared to the control.

This shows that the coating materials reduce seed germination and vigor, and this effect is intensified during storage. It is observed that the coating process reduced on average of 25% the germinations at time zero, and also occurred an average decrease of 25% of germination throughout the period of 120 days of storage. This suggests that other materials or combinations of materials should be tested in order to minimize such negative effects. It is observed that the coating, independently of the treatment product, negatively modified the physiological quality of the seeds during storage. Similar results were also observed by Roos (1979), Silva (1997), Silva and Nakagawa (1998), and Pereira et al. (2001). They found that seed coating may reduce the seed storage potential. Kim et al. (2000), studying different types of coating, among them the pelletizing, on the physiological quality of lettuce seeds, during nine months of storage, verified losses in germination and vigor. Pereira et al. (2004) also found losses in the physiological quality of pelleted seeds of *Brachiaria decumbens* during 12 months of storage.

Regarding the use of fungicide, Oliveira et al. (2003), studying different pelleting materials with or without fungicide in tomato seeds (*Lycopersicon esculentum* L.) during 24 months of storage, found that the fungicide associated with pelleting materials reduced the physiological quality of seeds.The physiological quality of coated seeds during storage may have been influenced by the climatic conditions in which they were stored. Roos (1979), studying different temperature and relative humidity conditions combined with four filler materials during storage in lettuce seeds, found that temperatures

lower than 10% and relative humidity less than 70% retained the physiological quality of these seeds for up to three years. The same author verified that the relative humidity above 70% and the temperature rises to 21°C caused a faster deterioration of the seeds as compared to non-coated seeds.

Another factor that may have negatively affected the physiological quality of the coated seeds was that they had higher water contents. This probably caused an increase in the respiratory rate of the seeds leading to loss of germinative potential. Different behavior was observed in the control, where the water contents of the seeds were inferior to the coated seeds.

Conclusion

Based on the results it can be concluded that Colza seeds coated with bentonite + fungicide have higher water contents, followed by bentonite + plant extract, bentonite + PVA glue and non-coated seeds; the water content of the seeds, independently of the filler material and treatment product are little influenced during the storage period. Coated seeds have the germination and vigor reduced more rapidly during storage. The bentonite + fungicide used for coating of colza seeds totally inhibits germination at 120 days of storage. Non-coated seeds statistically maintain germination at 120 days of storage. The third counting indicates further studies of longer periods for germination of coated colza seeds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Weed flora survey in field crops of Northwestern Ethiopia

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A survey was conducted in different field crops of six districts (Chilga, Gondarzuria, Metema, Takussa, Dabat and Dembia) in Northwestern Ethiopia within 2016 and 2017 cropping seasons. The objective of the study was to identify the most important weed species and to determine their frequency, density and uniformity at different altitudes. A quantitative and qualitative method was employed for the enumeration and identification of weed species. Quadrats were laid along transects and individual weed species in each quadrat was identified and counted. Frequency, uniformity, similarity index and relative abundance were used to determine the weed community structure. A total of 76 weed species belonging to 65 genera within 27 families were identified across different field crops. The most dominant families, based on the family dominance index (FDI), were Asteraceae, Poaceae, and Fabaceae with 78.97, 63.76, and 20.72 FDI, respectively. The most frequent, abundant and dominant weed species were *Digitaria abyssinica* (Hochst. Ex A. Rich), *Cyperus rotundus* L. and *Cynodon dactylon* L. The average values for frequency and dominance of weed species in arable fields ranged between 49.34 to 59.87% and 3 to 3.69%, respectively. Results obtained from this study would be useful in creating a weed management programme and making inform decision on choice of herbicides and directing research toward new or improved weed control measures.

Key words: Altitude, density, district, dominance, family, frequency, species.

INTRODUCTION

Weeds are one of the major constraints to crop cultivation that can affect crop yield based on their species composition and density (Kropff et al., 1992). Weeds compete with cultivated food crops for limited resources such as water, nutrients, and light. Weed competition reduces yield and consequently farm income (Hassannejad and Porheidar-Ghafarbi, 2012). Weeds infestation also encourage disease problems, serve as alternate host for deleterious insects and diseases, slow down harvesting, restricting operations, increase the cost of production, reduce the market value of crops and increase the risk of fire in perennial crops, plantation and forest reserves (Palumbo, 2013; Tena et al., 2012).

Although most farmers are less concerned about the negative impact that weeds impose on their crop, study results indicate that weeds share up to 45% of the total annual losses of agricultural products (Upadhyay et al., 2011).

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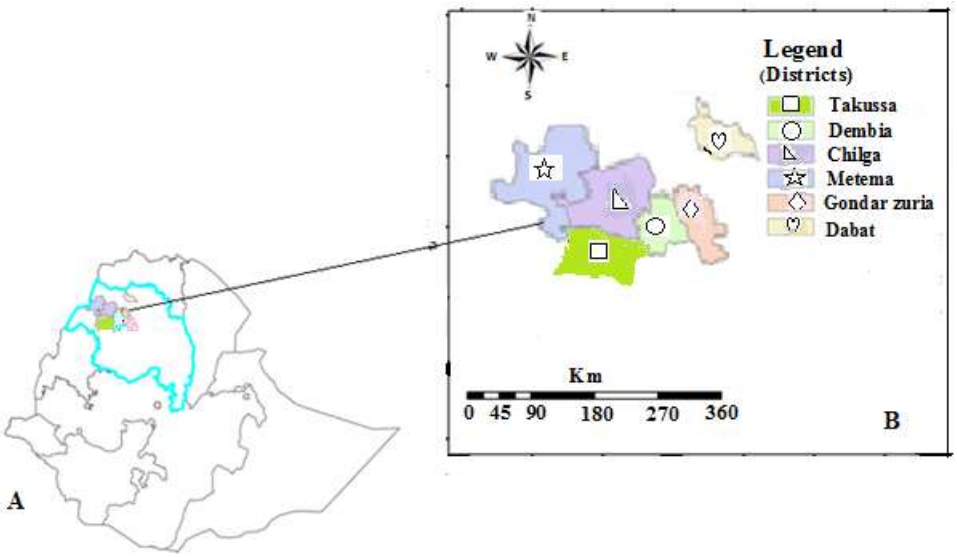


Figure 1. Map of weed flora surveyed districts in northwestern Ethiopia (A= Map of Ethiopia; B=Map of surveyed districts).

Yield losses due to weed competition in Africa range between 55 and 90% for maize, 50% for common bean, 40 and 80% for sorghum, 40 and 60% for cowpea, 50 and 100% for rice, 80% for cotton, 50 and 80% for wheat and groundnut, and 90% for cassava (Akobundu, 1987; Chikoye et al., 2004). On average it has been estimated that weeds cause yield losses of about 10% in the less developed countries and 25% in the least developed countries (Akobundu, 1987). Currently weeds are playing a significant role in making pest problems very complex. One of the main constraints faced by farmers in the production of arable crops is effective weed control (Vissoh et al., 2004).

Weed flora composition is strongly associated with regional climate, soil characteristics, and management methods (Dale et al., 1992; Salonen, 1993). Weed assessments are useful for determining the relative importance of weed species in farmlands (Thomas 1985; Frick and Thomas, 1992). Previously some studies have been conducted on weed flora and their distribution in Ethiopia (Stroud and Parker, 1989; Mesfin Tadesse et al., 2004; Ermias 2011), in Eastern Harerge (Tamado and Milberg, 2000), in mid-rift-valley of Ethiopia (Ayana, 2018), and in Southwestern Ethiopia (Getachew et al., 2018). However, detailed information about the distribution, occurrence and quantitative assessment of the weed flora of Northwestern Ethiopia field crops is lacking. Occasionally, as a result of scattered research projects in a given area there often is no coordinated effort to summarize the already existing knowledge and data in a common database for a particular district area. Specifically, agronomic practices and environmental fluctuations of a particular cropping area may affect weed community dynamics (Smith and Gross, 2006). Excessive weed growth is one of the most serious problems facing farmers throughout the tropics. There is a need for professionally trained weed scientists to become involved in research at the highest level, where existing technologies can be further developed and new ones generated (Akobundu, 1987). In addition to weed species distribution, it provides baseline information for monitoring changes in weed populations over time.

Surveys provide weed biologists and ecologists with quantitative information on weed communities that is used in the development of integrated weed management strategies, and weed scientists and extension specialists with information for weed control recommendations. Furthermore, the relative importance of common weed species in the major crops and cropping systems is not well documented (Stroud and Parker, 1989), especially in surveyed districts. Thus, knowledge of the weed community structure is an important component of weed management, and is essential in setting priorities for both weed management and research. Thus, the aims of this study were to identify the most important residual weed species in the northwestern area and determine the weed species' composition related to their distribution, density and uniformity.

MATERIALS AND METHODS

Description of the area

The weed flora survey was conducted in six districts (Chilga, Dembia, Gondarzuria, Dabat, Metema and Takussa) from lowland up to highland of the different agro-ecological zones of northwestern Ethiopia during 2016 and 2017 cropping seasons (Figure 1). These districts are different climatic areas of the northwestern part of Ethiopia with temperature ranging from 10 to

42°C and an annual rainfall ranging from 200 to 2200 mm (Menberu Teshome, 2017). The survey of surface topography is generally low from a low of 580 m and rising to highland areas in the northern areas with the highest point of 2700 m. The surveyed districts are major production areas of sorghum, teff, maize, wheat, barley and pulse crops. The weed samples were collected from the altitude range of Metema (580-1500 masl), Dabat (2100-2685 masl), Takussa (1600-1800 masl), Dembia (1750-1900 masl), Gondarzuria (1933-2700 masl), and Chilga (1850-2400 masl) districts (Assefa et al., 2018). The weed survey covered the major agricultural zones in Northwestern Ethiopia from Kolla at altitudes below 1800 masl, Weyna Dega (medium altitude) for altitudes between 1800 and 2400 m and Dega (high altitude) for areas above 2400 m (Hans, 1998).

Sampling procedure

The number of fields was selected in accordance with the proportion of the field crop area (cereals, niger seed and pulse crops) for each district. A stratified random sampling procedure (Cochran, 1977) was used to select fields. Weed flora data were collected from the first year in 82 and the second year in 70 crop fields (total of 152 crop fields). Weed vegetation in the field was sampled in 1 m^2 plots, located equidistant along a 'W' pattern consisting of five quadrants in each field following the methodology of Thomas (1985). The number of weed species in each plot was counted (density and cover percentage), and recorded for subsequent data entry and analysis. During data collection for unknown weed, specimens were given the code number and collected for further identification using the help of flora of Ethiopia (Stroud and Parker, 1989; Mesfin Tadesse et al., 2004) and also several handbooks were consulted to aid identification in the field. Data on the weed species at different districts were collected from the farmer fields and altitude of each field was measured with Geographic Position Service (GPS).

This sampling time was chosen because, most of the weeds were well established, most of them were in flowering or seed-setting stages, most of the annual weeds were only observed before the first harvest. Data for the two survey years of weed surveys were combined and summarized using three quantitative measures. These were frequency, mean field uniformity, and mean field density, which were computed for each species using the method of Thomas (1985).

Data analysis

Family dominance index (FDI) was counted following the methodology of Hassannejad and Porheidar-Ghafarbi (2012) to compare the relative contribution of each taxonomic family to weed species composition. It was calculated as the sum of the relative diversity, relative density, and relative coverage as follows:

$$\text{Relative diversity} = \frac{\text{Number of species in family}}{\text{Total number of species}} \times 100 \quad (1)$$

$$\text{Relative diversity} = \frac{\text{Number of individuals in family}}{\text{Total number of individuals}} \times 100 \quad (2)$$

$$\text{Relative diversity} = \frac{\text{Coverage of individuals in family}}{\text{Total coverage of individuals}} \times 100 \quad (3)$$

$$\text{FDI} = \text{Relative diversity} + \text{Relative density} + \text{Relative coverage} \quad (4)$$

The data on weed species were summarized using:

Frequency - Number of fields in which a species occurred (5)

expressed as percentage of the total number of fields surveyed; and

Field uniformity - Number of sampling quadrats in which a species occurred in a field (6)

expressed as percentage of the total number of samples (Tamado and Milberg, 2000).

The study sites were selected based on the local importance of weed species and the severity of the threats by the weed on the local diversities and accessibility of the study site. Density of weed species was estimation applying the following formulas (Arpana, 2013).

$$\text{Absolute density (AD)} = \frac{\text{Total number of individuals of a species in all quadrates}}{\text{Total number of quadrates}} \quad (7)$$

$$\text{Relative density (RD) (\%)} = \frac{\text{Absolute density for a species}}{\text{Total absolute density for all species}} \times 100 \quad (8)$$

The qualitative (species) and quantitative structure of weed communities were compared using the Sorensen similarity index (SSI) (Magdalena et al., 2013):

$$\text{SSI} = 2c \times 100 \times (a + b)^{-1} \quad (9)$$

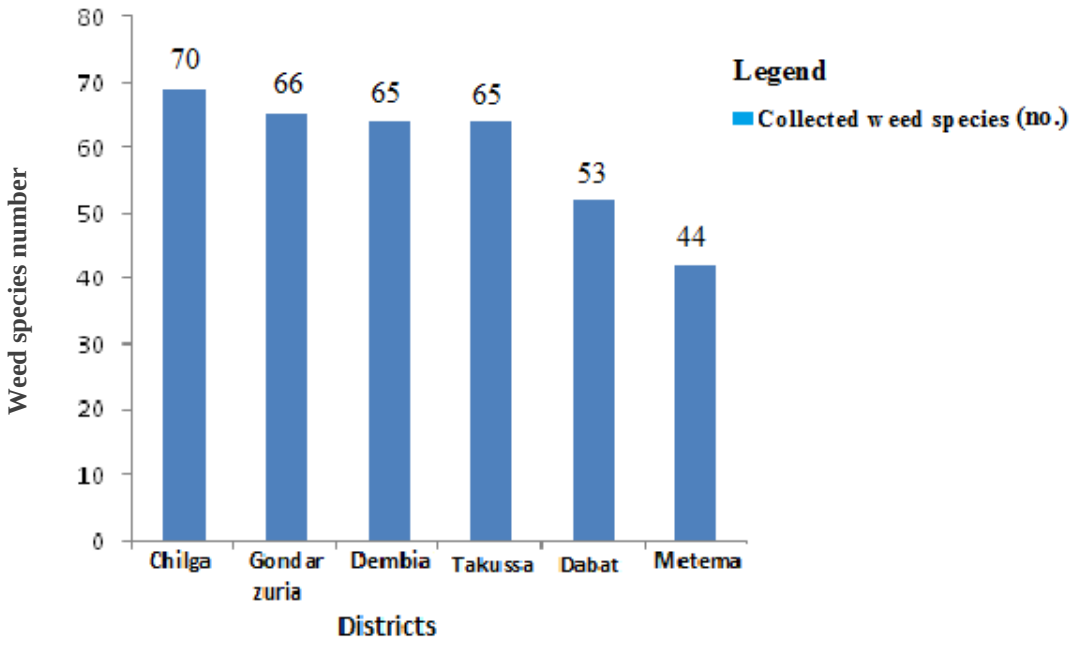
where c - total number of species shared by the two communities or total abundance of species shared by the two communities, a - number of species or total abundance of species in the first community, b - number of species or total abundance of species in the second community.

Similarity index showed the similarity of weed species composition among different districts.

RESULTS

Weed species across location

A total of 76 weed species from 65 genera were recorded on six districts (Metema, Dembia, Takussa, Chilga, Gondarzuria and Dabat) from farmer crop fields during 2016 and 2017 cropping seasons (Table 2). The highest number of weed species (70) was collected from Chilga followed by Gondarzuria district during survey period, where the altitude has similar ranges. The lowest number of weed species (44) was collected from lowland of Metema district, however, from the highest surveyed altitude range of Dabat district was collected 53 species out of the total collected weed species (Figure 2). List of weed *Avena fatua* L., *Galinsoga parviflora* Cav*., Bidens pilosa* L*., Medicago polymorpha* L. *Lolium temulentum* L.,



Fugure 2. The amount of collected weed species across different districts.

Table 1. Similarity index of weed species in six districts of northwestern Ethiopia.

District	Metema	Dabat	Takussa	Dembia	Gondarzuria	Chilga
Metema	100	47.42	75.22	73.40	61.81	57.90
Dabat		100	72.88	71.18	87.39	82.92
Takussa			100	92.31	88.55	85.92
Dembia				100	87.02	87.41
Gondarzuria					100	83.82
Chilga						100

Rumex nepalensis Spreng., *Sporobolus africanus* (Poir.) and *Chrysanthemum segetum* L. were typical highland species, while *Portulaca oleraceae* L., *Parthenium hysterophorus*, *Corchorus olitorius L., Boerhavia erecta* L., *Xanthium abyssinicum* Wallroth, *Heliotropium zeylanicum* (Burm.) Lam., *Urochloa panicoides* P. Beauv., and *Euphorbia hirta* were typical lowland species. Commonly wide spread weeds such as *Senna didymobotrya (*Fresen*.)* Irwin & Barneby, *Polygonum barbatum* L., *Oxygonum sinuatum* (Meissn.) Dammer, *Oxalis latifolia* H. B. K. *Ocimum basilicum* L.*, Nicandra physalodes* (L.) Gaertn., and *Galium hamatum* L. were the frequent recorded intermediate altitude species (Table 2).

Similarity index of weed species

The result showed a Sorensen similarity index (SSI) value of 57.90-87.41%, 61.81-88.55%, 71.18-92.31%, 72.88-75.22% and 47.42% among the districts of Chilga, Gondarzuria, Dembia, Takussa, and Dabat, respectively. Weed communities of crops grown in Chilga with Dabat, Gondarzuria, Takussa and Dembia districts were more similar with regard to species composition (SSI of 82.92-87.41%). While weed species composition was mainly dissimilar between Metema with Dabat and Chilga districts. Metema district of weed communities with lower altitude of Dembia and Takussa districts revealed substantially higher similarity with respect to floristic composition of 73.40 and 75.22% SSI than higher altitude of Dabat and Chilga districts 47.42 and 57.90%, respectively (Table 1).

Weed flora of crop fields

Among a total of 76 weed species, seven aggressive species *Digitaria abyssinica*, *Parthenium hysterophorus*, *Cynodon dactylon*, *Cyperus rotundus*, *Tagetus minuta*,

Table 2. Description of frequency, density and altitude of weed species on crop fields in Northwestern Ethiopia.

Weed species	Family	Altitude (m)	Frequency (%)	Uniformity (%)	AD (No.)	RD (%)
Acanthospermum hispidium DC.	Asteraceae	600-1800	19.08	10	0.31	0.55
Acanthus eminens (C.B.Clar.)	Acanthaceae	1500-2800	5.92	1	0.12	0.21
Ageratum conyzoides L.	Asteraceae	1900-2400	6.58	2	0.18	0.32
Amaranthus graecizans L.	Amaranthaceae	1000-1700	11.84	5	1.23	2.16
Amaranthus hybridus L.	Amaranthaceae	350-2350	13.82	6	0.15	0.26
Amaranthus spinosus L.	Amaranthaceae	400-2400	11.84	5	1.54	2.71
Argemone mexicana L.	Papaveraceae	150- 2500	2.63	1	0.12	0.21
Argemone ochroleuca Sweet	Papaveraceae	150- 2500	15.13	4	1.23	2.16
Avena fatua L.	Poaceae	2700-3200	1.97	2	0.13	0.23
Bidens pilosa L.	Asteraceae	750-2400	31.58	17	1.14	2.00
Boerhavia erecta L.	Nyctaginaceae	500-1700	3.95	3	0.26	0.46
Carduus nyassanus (S.Moore) R.E.	Asteraceae	2000-3500	21.05	10	0.17	0.30
Chenopodium album L.	Chenopodiaceae	1250-2350	4.61	2	1.23	2.16
Chenopodium opulifolium Schr.	Chenopodiaceae	1000-2250	15.79	4	0.20	0.35
Chrysanthemum segetum L.	Asteraceae	2000-2440	11.84	2	0.35	0.61
Cirsium vulgare (Savi) Ten.	Asteraceae	1900-3000	17.76	3	1.50	2.63
Commelina benghalensis L.	Commelinaceae	400-2700	36.84	25	1.20	2.11
Convolvulus arvensis L.	Convolvulaceae	1600-2400	3.95	3	0.18	0.32
Conyza bonariensis (L.) Cronq.	Asteraceae	1290-2600	7.89	4	0.87	1.53
Corchorus olitorius L.	Tiliaceae	250-1750	12.5	3	0.40	0.70
Crotalaria laburnifolia L.	Fabaceae	1300-1700	17.11	3	1.45	2.55
Cuscuta campestris Yuncker	Convolvulaceae	200-2350	5.92	2	0.86	1.51
Cynodon dactylon (L.) Pers.	Poacaea	1600-2700	52.63	36	1.71	3.00
Cynodon nlemfuensis Vandryst	Poaceae	200- 2400	38.16	20	1.35	2.37
Cyperus esculentus L.	Cyperaceae	200- 2500	11.18	8	1.23	2.16
Cyperus rotundus L.	Cyperaceae	250- 2500	49.34	28	2.00	3.51
Dactyloctenium aegyptium (L.) W.	Poacaea	150-2400	2.63	1	0.66	1.16
Datura stramonium L.	Solanaceae	600-2800	29.61	13	1.14	2.00
Digitaria abyssinica (Hochst. Ex A.Rich) Stapf	Poacaea	1400-2700	59.87	38	2.10	3.69
Echinochloa colona (L.) Link	Poacaea	250-2400	2.63	1	0.35	0.61
Eleusine indica (L.) Gaertn.	Poacaea	500-2800	2.63	7	0.87	1.53
Euphorbia hirta L.	Euphorbiaceae	240-2050	15.13	8	0.75	1.32
Galinsoga parviflora Cav.	Asteraceae	950-2800	25.66	20	1.22	2.14
Galium hamatum L.	Rubiaceae	1700-3600	3.95	1	0.15	0.26
Guzotia scabra (Vis.) Chiov.	Asteraceae	1500-3000	29.61	18	1.12	1.97
Heliotropium zeylanicum (Burm)L	Boraginaceae	300- 1900	29.61	13	0.75	1.32
Hibiscus trionum L.	Malvaceae	1200-2800	9.87	4	0.55	0.97
Hygrophila auriculata (Schum.) H.	Acanthaceae	500- 2800	11.84	3	0.32	0.56
Hyparrhenia rufa (Nees) Stapf	Poacaea	1200-2100	15.79	5	1.32	2.32
Ipomoea eriocarpa R. Br.	Convolvulaceae	500-1700	16.45	7	0.07	0.12
Lantana camara L.	Verbenaceae	500-2500	7.89	2	0.28	0.49
Launaea cornuta(Oliv. & Hiern) C. J	Asteraceae	400-1900	25	12	1.12	1.97
Launaea intybacea (Jacq.) Beauv.	Asteraceae	180-2300	23.03	13	0.75	1.32
Leucas martinicesis (Jacq.) R. Br.	Lamiaceae	200-2500	28.29	15	1.33	2.34
Lolium temulentum L.	Poacaea	2000-2900	25.66	12	0.54	0.95
Medicago polymorpha L.	Fabaceae	1400-3000	31.58	19	1.62	2.85
Nicandra physalodes (L.) Gaertn.	Solanaceae	600-2100	17.76	10	0.35	0.61
Ocimum basilicum L.	Lamiaceae	1000-2600	23.68	11	1.25	2.19
Orobanche spp.	Orobanchaceae	200-3000	2.63	1	0.25	0.44
Oxalis latifolia H. B. K.	Oxalidaceae	1700-2500	3.29	1	0.34	0.60

Table 2. Contd.

Oxygonum sinuatum (Meissn.) Dam	Polygonaceae	600-2500	27.63	15	1.00	1.76
Parthenium hysterophorus L.	Asteraceae	900-1800	46.71	35	1.68	2.95
Phalaris paradoxa L.	Poacaea	1800-2400	15.13	7	0.80	1.40
Plantago lanceolata L.	Plantaginaceae	1200-3200	13.16	5	0.72	1.27
Polygonum barbatum L.	Polygonaceae	1300-3000	9.21	2	0.18	0.32
Polygonum nepalensis Spreng.	Polygonaceae	1350-3200	10.53	3	0.23	0.4
Portulaca oleracea L.	Portulacaceae	150-2350	12.5	6	0.33	0.58
Rumex nepalensis Spreng.	Polygonaceae	1200-3900	7.89	3	0.36	0.63
Senna didymobotrya (Fresen.) Irwin & Barneby	Fabaceae	1450-2400	21.71	13	1.35	2.37
Senna occidentalis (L.) Link	Fabaceae	250-2400	9.87	4	0.34	0.60
Setaria verticillata (L.) Beauv.	Poacaea	100-1700	11.84	4	1.23	2.16
Snowdenia polystachya (Fresen)Pilg.	Poacaea	1500-2700	14.47	6	0.70	1.23
Solanum incanum L.	Solanaceae	150-2100	17.76	4	0.37	0.65
Solanum nigrum L.	Solanaceae	500-2300	12.5	5	0.76	1.34
Sonchus asper (L.) Hill	Asteraceae	1050-2850	21.71	10	0.89	1.56
Sorghum arundinaceam (Desv.)	Poacaea	600-2400	2.63	1	0.32	0.56
Sporobolus africanus (Poir.)	Poaceae	1500-2900	4.61	3	0.17	0.30
Stephania abyssinica (Dillon & A. Rich.) Walp.	Menispermaceae	1450-3400	1.97	1	0.87	1.53
Striga hermonthica (Del.) Benth.	Scrophulariaceae	550-1800	3.29	2	0.80	1.40
Tagetus minuta L.	Asteraceae	1350-2200	41.45	21	1.22	2.14
Tribulus terrestris L.	Zygophyllaceae	near masl	17.76	7	0.75	1.32
Trichodesma zeylanicum (L.) R.	Boraginaceae	500-2000	11.84	5	0.11	0.19
Urochloa panicoides P. Beauv.	Poaceae	600-1900	11.18	4	0.12	0.21
Vernonia karaguensis Oliv & Hiern	Asteraceae	1400-2750	23.68	8	0.65	1.14
Xanthium spinosum L.	Asteraceae	1700-2600	5.92	3	0.75	1.32
Xanthium strumarium	Asteraceae	900-2000	28.95	13	0.70	1.23

AD: Absolute density; RD: elative density; no = number of individual species/quadrant.

Cynodon nlemfuensis and *Commelina benghalensis* were recorded and widely distributed with higher than 35% frequency value while the lower than 10% frequency value was recorded from 25 weed species on surveyed crop fields. The ranking of weeds based on frequency and field uniformity was slightly different (Table 2). The species that had the highest frequency and largest uniformity 38% was *D. abyssinica* and followed by 36% of *C. dactylon* among all surveyed weed flora. Hence, these species were much more frequently recorded and uniformly distributed in fields than other species. The lowest frequency with the lowest uniformity was observed on *Stephania abyssinica* weed species (Table 2).

The absolute density (AD) value of the species varied from 0.11 to 2.10 plants/m^2. The highest abundance value (2.10 plants/m^2) was recorded by *D. abyssinica* Stapf followed by *C. dactylon* (L.) Pers. (1.71 plants/m^2), *P. hysterophorus* L. (1.68 plants/m^2) and *Amharanthus spinosus L*. (1.54 plants/m^2). Whereas, the least abundance value (0.11 plants m^2) was recorded from *Trichodesma zeylanicum* (L.) R. weed species (Table 2). Results of this study showed that *D. abyssinica*, *C. rotundus*, *C. dactylon* and *P. hysterophorus* were in the top ranking of weed species in crop fields according to relative density (RD) 3.69, 3.51, 3.00 and 2.95%, respectively. While the least RD percentage was indicated by *Ipomoea eriocarpa* and *Trichodesma zeylanicum* weed species (Table 2). Among weed species, *D. abyssinica* Stapf (59.87 and 3.69%), *C. dactylon* Pers. (52.63 and 3.00%), *P. hysterophorus* L. (46.71 and 2.95%), and *C. benghalensis* L. (36.84 and 2.11%) of frequency value, and of dominance values were recorded on weed species from surveyed district crop fields, respectively (Table 2). Mainly the weed species with the higher frequency value was higher relative density value, however, in some cases, the higher frequency value showed lower relative density value when compared with one to the other weed species. The frequency percentage of *Chenopodium opulifolium* Schr *(*15.79%) was greater than the value for *Corchorus olitorius L.* (12.50%); however, the relative density of *C. olitorius L.* was greater than *C. opulifolium* Schr. and had values of 0.70 and 0.35%, respectively, conclusively *C. olitorius L* was twice as dominant as *C. opulifolium* Schr. weed species. Dominance of perennial weed such as *D. abyssinica*, *C. rotundus* L, *P. hysterophorus, C. dactylon* (L.) Pers., and *Cirsium vulgare* (Savi) Ten. were aggressively wide spread since

Table 3. Family dominance index of weed flora survey in northwestern Ethiopia.

No.	Family	Species number	Relative diversity (%)	Relative density (%)	Relative coverage (%)	FDI
1	Acanthaceae	2	2.63	0.77	0.65	4.05
2	Amaranthaceae	3	3.95	5.13	1.96	11.04
3	Asteraceae	17	22.34	25.68	30.95	78.97
4	Boraginaceae	2	2.63	1.51	2.72	6.86
5	Chenopodiaceae	2	2.63	2.51	1.31	6.45
6	Commelinaceae	1	1.32	2.11	3.77	7.20
7	Convolvulaceae	3	3.95	1.95	1.61	7.51
8	Cyperaceae	2	2.63	5.67	5.44	13.74
9	Euphorbiaceae	1	1.32	1.32	1.21	3.85
10	Fabaceae	4	5.26	8.36	7.10	20.72
11	Lamiaceae	2	2.63	4.53	3.98	11.14
12	Malvaceae	1	1.32	0.97	0.60	2.89
13	Menispermaceae	1	1.32	0.14	0.50	1.96
14	Nyctaginaceae	1	1.32	0.46	0.60	2.38
15	Orobanchaceae	1	1.32	0.44	0.30	2.06
16	Oxalidaceae	1	1.32	0.60	0.20	2.12
17	Papaveraceae	2	2.63	2.37	1.91	6.91
18	Plantaginaceae	1	1.32	1.27	0.91	3.50
19	Poacaea	15	19.74	21.73	22.29	63.76
20	Polygonaceae	4	5.26	3.11	3.62	11.99
21	Portulacaceae	1	1.32	0.58	0.91	2.81
22	Rubiaceae	1	1.32	0.26	0.20	1.78
23	Scrophulariaceae	1	1.32	1.40	0.30	3.02
24	Solanaceae	4	5.26	4.60	4.88	14.74
25	Tiliaceae	1	1.32	0.70	0.70	2.72
26	Verbenaceae	1	1.32	0.49	0.30	2.11
27	Zygophyllaceae	1	1.32	1.32	1.06	3.70

FDI: Family dominance index.

these weed species grow over years on field crops and that can be reduced on crop yields. Among perennial weed species, *C. rotundus* L and *P. hysterophorus* was observed in the dominance of 3.51 and 2.95% of value on field crops (Table 2).

Family dominance index (FDI)

A total of 76 weed species belonging to 65 genera and 27 families were recorded. Asteraceae and Poaceae were recorded as dominant families and contained a total of 17 and 15 weed species, respectively. Species richness and diversity were generally higher during the cropping season of July up to December. Among families, the highest relative diversity of 22.34% was recorded from Asteraceae and followed by 19.74% on Poaceae family. While the least relative diversity, one species was recorded from individual family of fourteen different families (Table 3).

Relative density varied among weed families. The highest relative densities of 25.68 and 21.73% were recorded for Asteraceae and Poaceae families, respectively (Table 3). While the third relative density 8.36% was obtained from Fabaceae family. Consistently, the relative coverage 30.95, 22.29 and 7.10% were also the greater on Asteraceae and Poaceae family and followed by the family of Fabaceae, which covered the wide range of farming fields among the weed families. The main dominant plant families were Asteraceae, Poaceae, and Fabaceae with 78.97, 63.76, and 20.72 FDI, respectively. These implied that families had the higher density and coverage in the crop field areas (Table 3). However, the lowest relative density (0.14%) and dominance (1.78) was recorded from Menispermaceae and Rubiaceae family, respectively, where the species were found occasionally from farm fields (Table 3).

DISCUSSION

The result indicated that the occurrence of weed flora in

medium altitude range had more weed species diversity than the highest and the lowest altitude location. In most cases, the high altitude weed species diversity in fields was more similar with medium altitude than the corresponding of lower altitude weed flora. As described by Kropff and Spitters (1991), if the similarity index is below 60%, it is said to be that the two locations have different weed communities. Since similarity index for the different location were greater than 60%, it can be concluded that the locations exhibited similar weed community. The results showed that the highest similarity index was obtained between Dembia and Takussa districts from both medium altitudes, while the lowest similarity was obtained between Dabat and Metema followed by Chilga and Metema districts, and with comparison of higher with lower altitude area ranges. The SSI result indicated that due to the agro-climatic condition of Metema was more related to Dembia and Takussa district than higher land agro-climatic condition of Dabat and Chilga districts. The reason behind that was due to the altitude range represents a complex gradient and influences other environmental and crop management variables (Tamado and Milberg, 2000). Therefore, it can be designed the strategic plan of similar management options.

The top-ranking weeds species *D. abyssinica*, *C. rotundus*, *C. dactylon* and *P. hysterophorus* were the most aggressive and difficult weeds to control in different surveyed areas. High frequency of these weeds showed that they are a serious problem in all agricultural fields. RD was used for quantitatively ranking of weed species and these were recorded with maximum weed species dominance. Holm et al. (1977) showed that *D. abyssinica* is considered one of the most troublesome crop weeds in Ethiopia, Kenya, Uganda and Tanzania. Heavy infestations can kill coffee bushes, reduce sisal yields by 2 t/ha and cotton yields by 50%. *C. rotundus* is one of the most invasive weeds known, having spread out to a worldwide distribution in tropical and temperate regions and it has been called "the world's worst weed" (Belachew and Tessema, 2015; Tena et al., 2012). In addition, these two weed species (*C. rotundus* and *P. hysterophorus*) were producing allelo-chemicals that retarded the crop growth and further reduced yields. Similar results were found from Roger et al. (2015) and reported that if the specific plant species had higher frequency and dominance value, it indicates the economic importance of it. Therefore, this study confirmed that these aggressive weed species are the major social, environmental and economic threats in the study area. Special attention and strategic plan required for the management of these aggressive weed species.

This survey revealed that *P. hysterophorus* and *C. rotundus* are becoming a problem of cropping areas. The allelochemicals released from *P. hysterophorus* inhibit the growth of pasture grasses, legumes, cereals, vegetables, other weeds, and even trees (Arpana, 2013). Manual control of parthenium weed by farmers resulted in some of them developing skin allergies, itching, fever, and asthma. *P. hysterophorus* can affect crop production, animal production and human health and also suppress the associated species through the release of allelochemicals from decomposing biomass and root exudates. Weed species such as *P. hysterophorus* and *C. rotundus* was reported to germinate throughout the summer and consequently late germinating plants are never controlled (McFadyen, 1992; Navie et al., 1996; Arpana, 2013).

Other aggressive weeds such as *A. hybridus, T. minuta,* and *B. pilosa* once their crop flowering stage have passed instead of removal of weeds, produced high number of seeds for the next cropping seasons (Chivinge, 1988). The result is that these weeds come up in larger numbers in the subsequent seasons, as more seeds will have been added to those already in the seed bank. If more seed production is coupled with weed seed dormancy then the problem is worsened. Though some weeds, such as *A. fatua* L., *A. mexicana L., D. aegyptium* (L.) Willd. and *S. abyssinica* (Dillon & A. Rich.) Walp. had low frequency, they were considered to be problematic weeds by farmers (Table 2). *A. fatua* L. and *Plantago lanceolata* L. were problematic weeds on barely and teff production areas, respectively.

Three parasitic weeds (*Striga hermonthica*, *Cuscuta campestris* and *Orobanche* spp.) were recorded on field survey. They were the most problematic in some parts of Gondarzuria, Metema and Takussa districts where these weeds occurred were forced to abandon growing sorghum, maize, niger seed and faba bean due to the plant parasite infestation. By far the most economically damaging parasites are *Striga* (witchweeds), *Orobanche* and *Cuscuta* species (Joel et al., 2007). Parasitic weed *Striga* spp. produces 400 to 500 seeds per capsule and seeds remain viable for 15 to 20 years (Ramaiah et al., 1984). The *Striga* spp. were more troublesome in lowland areas in which they commonly grown in maize and sorghum crops. The geographic distribution and the infestation level of *Striga* are steadily increasing, particularly in sub-Saharan Africa (Emechebe et al., 2004; Ejeta, 2007).

The relative higher score of FDI for Asteraceae, Poaceae and Fabaceae families were recorded due to the greater number of species and better adaptability under dominant environmentally conditions compared to other families. The survey result showed that the most important families in Northwestern Ethiopia based on the number of taxa and FDI were Asteraceae, Poaceae and Fabaceae families. These three families were also the most important in small-scale farming in Highland Peru, Central Mexico, Northern Zambia, Eastern and Southwestern Ethiopia (Tamado and Milberg, 2000; Getachew et al., 2018). These families are very species rich, so it is not surprising that they contain many weeds. The weed flora in crop fields of Northwestern Ethiopia

was dominated by few common species, which is a common phenomenon in extensive farming systems. *P. hysterophorus* as a noxious weed species in Asteraceae get top family in ranking than other families. So that these families were the most diverse family compared to other families. It seems that in arable lands due to continuous tillage, growth conditions are more favorable for annual weeds in comparison with perennial weeds (Hassannejad and Porheidar-Ghafarbi, 2012).

The most difficult and dominant weeds

These are plants that pose potentially serious threat to primary crop production or in farm lands (the most difficult weeds). The most difficult weeds to control in surveyed districts were *C. rotundus, C. dactylon, C. benghalensis*, *A. hispidium*, *S. hermonthica* and *P. hysterophorus* (Table 2). Especially most farmers reported that *C. rotundus* weed grew in dense stands and subsequent generations always came up soon after removal of one generation. *C. rotundus* was the most difficult weed even after weeding, it was also reproduced by vegetative and became the second most difficult weed to control in low altitude areas. Invasive species are concern because of their capability of spreading fast, their high competitiveness and ability to colonize new areas within short periods (Gualbert, 2013). The nature and severity of the impacts of these species on society, economic life, health and national heritage are of global concern (Mc Neely et al., 2001). Parthenium weed also causes severe human and animal health problems and agricultural losses (Arpana, 2013). *S. hermonthica* was found as a serious problem particularly in Gondarzuria, Metema, and Dembia districts with the heaviest infestations being on sorghum crop fields. Parasitizing important economic plants, *S. hermonthica*, weed is one of the most destructive pathogens in Africa (Agrios, 2005). In fact, *S. hermonthica* affects 40% of Africa's arable savanna region, resulting in up to $13 billion lost every year (Anonymous, 2009).

Some weed species have especial effect of crop production, *E. indica* produces numerous small seeds which germinate quite early in the crop-growing season and plants have a vigorous and extensive root system (Holm et al., 1977). Field observation during the survey revealed that once the weed was more than one and a half months old from the time of emergence, it became very difficult to remove manually or mechanically. In this case, *C. benghalensis* produces both aerial and subterranean seeds and also reproduces it vegetatively. If the weed is removed by hand or mechanically, stems break off and root at the nodes, producing new plants. Thus, weeding may indirectly multiply the plant. Budd (1975) found *C. benghalensis* to be among the top most aggressive weeds in the farm fields. Control at that stage would be difficult except probably by the use of chemicals. Over 30% of visited farmers' fields showed that weeds and crops of the same size before the first weeding was observed. This is too late to remove weeds as the detrimental effects of weeds on crop growth and final yield would have great loss. Most farmers tended not to remove weeds.

Specific area invasive weeds

The *Chrysanthemum segetum* L. was a serious weed species on barley and wheat crops in Dabat district and becoming a noxious weed to barely production area and currently became caused yield reduction, seed quality, highly widespread and dominant especially in barley production of northern high land areas.

Utilization of weeds by farmers

The amount of each weed collected from different crop fields and used for human usage was generally small, whereas the majority of grass species were used as cattle forage. Farmers collected not only weeds from the farm fields' ecosystem, but also cereal straws (as forage), grasses as house roof construction, Fabaceae straws, and shrubs as firewood. To feed cattle, crop straws and weeds in the farm fields were the most widely utilized resources by farmers in both the rainy and dry seasons, followed by grazing in crop fields after harvest. Thus, higher frequency (>35%) does not indicate the economic or sociological importance of a weed species, as some weeds have other uses, such as feed (*C. dactylon*, and *C. rotundus)* for livestock, which can be especially important in the lowlands.

Conclusion

The present weed survey cannot be assumed to be extensive and complete, but the study was to analyze the variation of weed frequency, uniformity, abundance and the identified species obtained represent a sufficient sample size to obtain basic information on current weed problems in northwestern farming system. The species list obtained is rather extensive and demonstrates that weeds are still a significant problem in agriculture system. Information about weed composition and their relationships with altitudes in each district would be beneficial in the selection of essential weed management methods and necessary to sufficiently describe the relative ranking of weeds. The most aggressive and difficult-to-control weeds in the survey have been identified in the survey at different altitudes. Weeds which have started increasing over the past few years are now known and a base for future weed surveys has been established. Whereas extensive field studies would be necessary to quantify the abundance and diversity of weeds, a survey is a fast and inexpensive approach

allowing one to cover a large area.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Study of diversity in some Moroccan population of saffron (*Crocus sativus* L.)

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To study Moroccan saffron germplasm variability relating to different agro-morphological and phenological traits, 969 saffron corms (accessions) were collected from thirteen different sites located in traditional saffron area of Taliouine-Taznakht. The study confirmed a wide range of phenotypic variability within and between populations. The variance analysis revealed that the mother corm weight (MCW), taken as covariant, has significant effect on all studied traits. The difference within and between origins (Provenances) was highly significant for all traits, which showed highly significant correlation. The flowers number (NF) as well as the number of daughter corms weighing above 7 g per plant (NDC≥7) turned out to be the most determinant parameters of saffron yield. The produced FN per corm varied from 1 to 9 with an average of 2.2 flowers. P1 population recorded a flowering rate of 65.5% with a maximum average of NF (2). Stigmat length (SL), which is an important yield trait, showed wide variation between origins from 32 to 38 mm. The mean stigma dry weight (DSW) varied from 4.2 to 6.2 mg with a maximum of 7.1 mg per flower recorded in P1. The PCA revealed 5 homogeneous main groups inside the studied populations. The first one was monoorganogenic and consisted of P1 population only, a group characterized by high values of MCW, NF, NDC≥7 and DSW. This study confirms as well a noticeable influence of corm origin on saffron yield, explained by the genotypic profile and/or the epigenetic effects of the different origins. These results proved a variability which should be useful to the selection program aiming the improvement of saffron productivity in Morocco.

Key words: Variability, agro-morphological traits, phenological traits, saffron corms, Morocco.

INTRODUCTION

Saffron (*Crocus sativus* L.) is one of the most important spice plants because of the pistil high value that grow inside its flowers. Growing in different countries under various soil-climate conditions, during many centuries, saffron has been influenced by various stressful factors and has undergone different sorts of mutations. Every changes and became a unique genotype, a new clone. Clones within a population grow together as a mixture, but they never combine into the same genetic structure because of their sterility (Agayev et al., 2012). Most

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Table 1. Details of saffron germplasm lines collected from 13 locaions of Morocco.

Origin code	District	Location (Douar)	Altitude (m)
P1	Askaouen	Askaouen	1900 - 2200
P2	Tawyalt	Assaka	1900 - 2200
P3	Tawyalt	Anrouz	1900 - 2200
P4	Sidi Hssaein	Tasgna	1450 - 1650
P5	Zagmouzen	Darf	1450 - 1650
P6	Zagmouzen	Darf	1450 - 1650
P7	Assaysse	Assaysse	1650 - 1900
P8	Zagmouzen	Darf	1450 - 1650
P9	Agadir melloul	Tamlakout	1650 - 1900
P15	Askaouen	Askaouen	1900 - 2200
P16	Askaouen	Askaouen	1900 - 2200
P17	Askaouen	Askaouen	1900 - 2200
P18	Askaouen	Askaouen	1900 - 2200

operating mutations and other genetic changes are preserved in the local populations (Singh et al., 2015). Such a process led to diverse clones with a high, medium or low viability potential. High potential clones are prospering but have small extent whereas the medium ones prevail in majority. The third group is exposed to a genetic decline leading to a partial disappearance and replacement by the first and the second group. That is the natural selection, self-renewal selection (Agayev and Zarifi 2010).

Saffron biological characteristics make its breeding too complicated (Agayev et al., 2012). Studies of the divergence are of great importance in the variation estimation and the potential of its future utilization in crop improvement programs (Salwee and Nehvi, 2014). Corm multiplication in saffron does not induce genome variation, except some natural mutations which are not easily detectable in triploid saffron specie (Salwee and Nehvi, 2014).

Moroccan saffron cultivation development depends mainly on the improvement of yields which appear to be relatively small and variable compared with other producing countries. The increase of yields requires an efficient cultivars selection. However, an evaluation of the existing population from different provenances is primordial as a preliminary step. Hence, the present work aims to study the genetic diversity of the Moroccan saffron populations relating to some agro-morphological and phenological traits.

MATERIALS AND METHODS

The saffron corms were collected in August 2014 from 13 representative villages of the main traditional saffron cultivation areas in Morocco (Table 1). Individual corm samples were identified through an access code, the name of the farmer, and plot location, and they were characterized through their diameter (cm) and weight (g). In the first year, all the collection was planted at the National Institute for Agricultural Research (NIAR) in the experimental station of Annoceur. This site is located in the province of Sefrou, in southern Fes-Meknes Region at an elevation of 1350 MAMSL and characterized by an annual average temperature ranging from -7 to 40°C and annual precipitation of 500 mm. Uniform cultural conditions were maintained for raising a good crop. Germplasm lines were planted in early October 2014 for their multiplication. In July 2015, daughter corms of each accession were harvested separately. Then, their number and weight were recorded. For a second multiplication cycle, the daughter corms were planted in September 2015 in a completely randomized design in Berrechid area, located on south of Casablanca at an altitude of 350 MAMSL with an annual temperature ranging from 5 to 32°C and annual precipitation of 365 mm. The soil is clay to sandy with an alkaline pH (8.35), a normal electric conductivity (0.15 mmhos/cm) and a high content of organic matter (2.78%).

The collection involved 969 corms from 13 origins. The number of accessions per population varied from a minimum of 16 corms to 155 as maximum. Corms were planted in rows at a depth of 10 cm with inter and intra row distance of 40×40 cm. During the growing season, twelve agro-morphological traits were studied; mother corm weight (g) (MCW), flowers number (FN), fresh stigma length (cm) (FSL), stigma dry weight (mg) (DSW), number of days to anthesis (days) (DA), flowering duration (days) (FD), number of days to 100% flowering (FED), number of sprouts (NS), plant height (cm) (PH), number of daughter corms (NDC), number of daughter corms over 7 g (NDC≥7), and number of daughter corms below 7 g (NDC<7).

Statistical analysis were carried out using descriptive statistics, analysis of variance (ANOVA) and principal component analysis (PCA) using Past 3.10 software and Minitab 17.1.0 software. Data was square root transformed to ensure normal distribution and homogeneity in variance and they were further analyzed by ANOVA. To study the structure of Moroccan saffron germplasm, distances between populations were computed using the Euclidean distances using previously standardized data. Clustering of the genotypes was performed using UPGMA clustering method.

RESULTS AND DISCUSSION

Descriptive analysis

The saffron accessions collected from 13 traditional saffron areas of Morocco were evaluated through twelve

Table 2. Descriptive statistical analysis of agro-morphological traits in Saffron (*Crocus sativus* L.).

Trait	N	MCW (Units)		FN (Units)		NS (Units)		PH (Units)		NDC (Units)		NDC<7 (Units)		NDC≥7 (Units)	
Orig.		**Avg**	**CV**	**Avg**	**CV**	**Avg**	**CV**	**Avg**	**CV**	**Avg**	**CV**	**Avg**	**CV**	**Avg**	**CV**
P1	58	22.3	25.8	**2.0**	107.0	7.2	40.3	**31.0**	22.5	8.2	31.4	5.1	54.3	**3.1**	50.4
P15	42	12.6	30.1	0.4	154.9	6.4	34.4	29.2	19.7	6.9	31.5	6.0	35.2	0.9	91.1
P16	64	14.1	28.5	0.6	178.9	5.8	**50.9**	28.2	27.6	5.9	40.1	4.2	58.7	1.7	89.7
P17	32	13.1	37.5	0.4	**251.2**	4.7	43.2	26.1	**35.1**	4.6	**47.9**	3.7	60.2	0.9	**107.6**
P18	15	12.7	34.1	0.5	139.4	4.7	44.8	27.9	23.0	5.8	34.0	4.4	49.2	1.3	96.8
P2	87	16.8	32.7	0.5	230.5	5.4	39.6	28.9	23.0	6.3	37.0	4.2	53.7	2.1	78.8
P3	16	12.8	35.5	0.4	165.1	5.5	49.7	30.3	21.0	5.8	39.3	4.9	46.1	0.9	82.3
P4	116	17.3	33.4	0.7	149.9	6.1	34.2	**31.0**	16.2	6.6	34.5	4.2	56.6	2.3	77.9
P5	127	**22.9**	34.0	0.7	195.1	7.4	44.1	28.6	26.6	8.5	38.4	5.9	50.8	2.7	78.7
P6	**155**	18.8	31.7	0.7	215.5	6.3	40.6	28.9	27.2	7.6	35.1	5.0	56.5	2.6	79.8
P7	60	19.5	31.2	0.9	179.2	5.8	30.7	28.9	20.7	6.1	33.3	3.9	53.4	2.2	81.9
P8	148	22.5	**38.9**	1.3	156.9	**7.8**	43.8	29.5	20.0	**8.9**	39.3	**6.5**	54.3	2.3	89.0
P9	49	19.1	36.7	1.4	140.9	5.0	45.2	27.4	28.8	6.7	44.7	4.6	**72.2**	2.0	78.1
Gen	969	18.9	38.4	0.9	180.7	6.4	44.2	20.1	23.8	7.3	40.7	5.0	57.7	2.2	84.5

Maximal values are bolded and minimal values are underlined.

Table 3. Contribution of mother corm weight towards total variance.

Factor	DF	SS%	F
Origin	12	20.98	21.15***
Error	956	79.02	-
Total	968	100.00	-

*, **, ***: significant at 0.05, 0.01 and 0.001, respectively.

agro-morphological traits. The mean values were subjected to descriptive statistics to evaluate the extent of the diversity within different sampled populations (Table 2).

The NF is the most polymorph character which recorded the highest CV (180%) whereas the plant height was less variable between populations (CV = 23.8%). Daughter corms number (NDC) representing a determinant productivity factor in the first year of saffron cultivation exhibited average CV over different populations to the extent of 40.7%.

Study confirmed a wide range of variability of agro-morphological traits between populations. The flowers number, the shoots number per corm and the daughter corms number ranged, respectively from 0.4 (P15) to 2.0 (P1), 4.7 (P17, P18) to 7.8 (P8), and 4.6 (P17) to 8.9 (P8). P1 recorded the maximum flower number and daughter corms number weighing above 7 g (3.1), whereas the least number of corms was recorded by P15 (0.9) (as shown in Table 2).

The behavior of different accessions from each provenance shows a different profile. Especially, P17 which recorded the highest CV for most of the characters. Thus, enhances a phenotypic variation inside P17. P1 recorded the lowest CV and less diversity between its accessions.

Previous studies about saffron diversity have shown variability in agro morphological traits. In fact, Sheikh et al. (2014) revealed significant genotypic differences for FN (0.8-1,96), NS (15.4-26.6), PH(23.2-35.6) and NDC (3.46-9.3) and highlighted the environment effect on the character expression. Singh et al. (2015) has studied 28 accessions from Cachemire and has reported a NF per corm ranged between 2 and 4.

Variance analysis

The variance analysis showed highly significant difference for MCW (Table 3). Results indicate the existence of specificities of each origin. The residual variation accounted for 79% of the total variance, showed high level of variation within the same origin, while the locations accounted for 21% of the total variance (Table 3). This high level of MCW variation can be explained by the difference within origins. In fact, MCW ranged from

Table 4. Analysis of covariance for different agro-morphological traits in saffron (*Crocus sativus* L.).

Factor	DF	FN	NS	PH	DCN	DCN < 7	DCN≥ 7
MCW	1	107.7***	164.0***	24.0***	174.1***	9.7**	228.5***
ORIGIN	12	4.4***	4.6***	2.2**	6.6***	6.8***	3.1***

*, **, ***: significant at 0.05, 0.01 and 0.001 respectively.

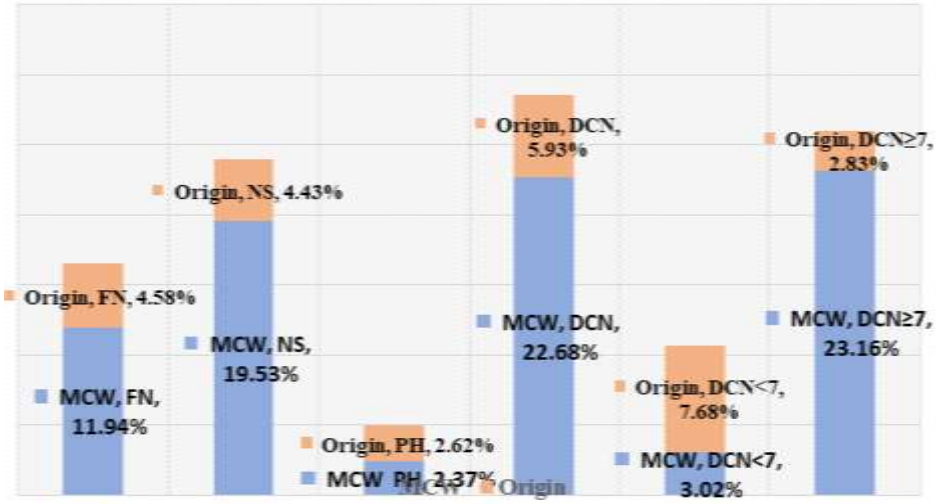

Figure 1. Contribution of mother corm weight and origin to total variance.

22.9 g for P5 to 12.5 g for P15.

Mother corm weight taken as covariant showed very high significant effect on most of the traits studied (Table 4).

Mother corms weight and origins effect

Contribution of the mother corm weight to the total performance of the daughter corms depends on the evaluated traits. The results confirmed a greater contribution of the mother corm weight to the total variability particularly the number of the daughter corms weighing above 7 g (Figure 1). In fact, the mother corm weight contributed by 23.16% to the total variance while the provenance contributed only by 2.83%. Similar results have also been confirmed by Soheilivand et al. (2007), which can be explained by the total reserves available to the production of new corms. For the number of shoots, MCW contributed 19.53% to the total variance while only 4.43% of the variance can be explained by the origin. Similar high contribution of the MCW compared to the origin has been observed for the total number of daughter corms and the number of flowers with 22.68% against 5.93% and 11.94% against 4.58%, respectively. On the other hand, the influence of the mother corm weight was relatively equivalent to that of the origin for the plant height, whereas, the influence of the origin on the total variability was pre-dominant for number of daughter corms weighing less than 7 g (7.68% vs. 3.02%). Several studies have highlighted the importance of the weight of the mother corms on the saffron production parameters like the number of flowers and the number of large daughter corms which are the two most economically important traits (Soheilivand et al., 2007; Agayev et al., 2012).

The differences between the origins were also highly significant for all the studied traits (Table 4). P1 population recorded an average more than 3 flowers per flowering corm coupled with 8 daughter corms (DCN) and 3 daughter corms weighing above 7 g/corm (DCN≥7) (Figures 2 to 5). The populations P5, P6, P8 and P9 were also close to P1. On the other hand, P3, P15, P17 and P18 populations were less productive. P3 produced a very low flower number (less than one flower/3 planted corms) with only 1 corm weighing above 7 g/mother corm.

The classification and grouping of the origins based on productivity traits (flowers number and daughter corms number) revealed the superiority of P1 and P8 (Tables 5 and 6). In terms of flowers number, P9 was observed to be as productive as P1, whereas for daughter corms number, P5 and P1 are observed to be as efficient as P8 (Tables 5 and 6). On the other hand, P17 and P3 were

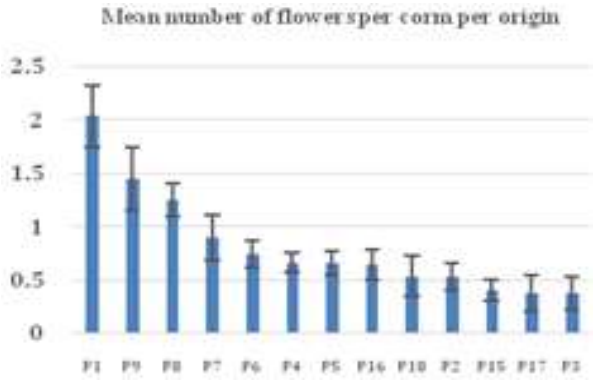

Figure 2. Distribution of flowers number (± standard deviation).

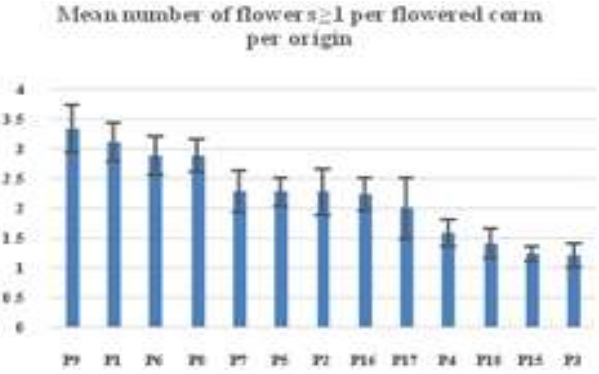

Figure 3. Distribution of flowers number ≥1 (± standard deviation).

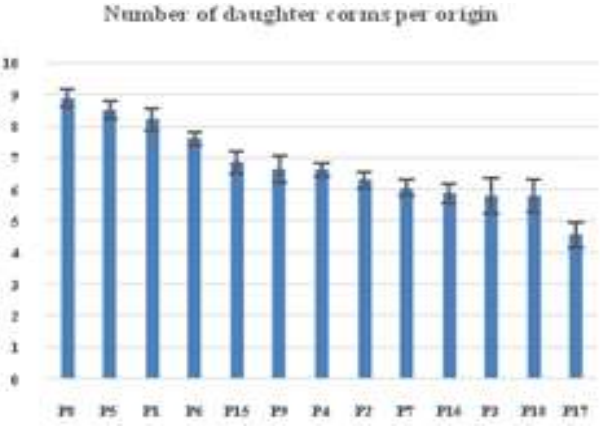

Figure 4. Distribution of daughter corm number (± standard deviation).

observed to be the less productive origins in terms of the flowers number and P17, and P18 in terms of the corms number produced

Ben El Caid et al. (2018) reported that regardless of number daugther corms, positive correlation was stated

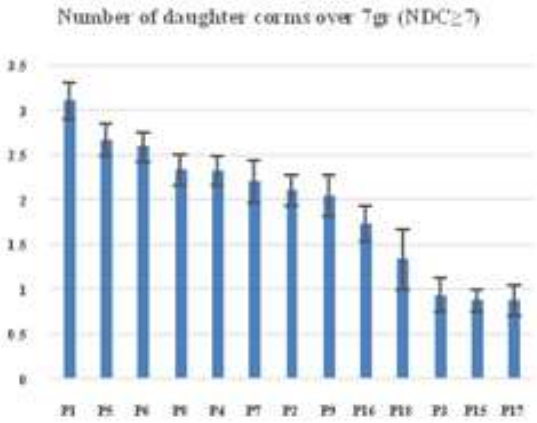

Figure 5. Distribution of daughter corm number ≥ 7 g(± standard deviation).

Table 5. Origins classification by the flowers number in saffron.

Original	Mean	Grouping
P1	**2.03**	**A**
P9	1.45	AB
P8	1.25	B
P7	0.90	BC
P6	0.74	BC
P4	0.66	BC
P5	0.65	BC
P16	0.64	BC
P18	0.53	BC
P2	0.53	BC
P15	0.40	BC
P17	0.38	BC
P3	0.38	BC

Values followed by a common letter within each column are not significantly different at P < 0.05 using the Duncan's Multiple Range Test (DMRT).

between the studied agro-morphometric traits and provenance Agadir Melloul in FSA planting site.

Correlations between traits

All the studied traits exhibited a highly significant correlation except the correlation between the corms number below 7 g and the flowers number (FN) per mother corm (Table 7). Apart from the correlations between dependent traits, NDC≥7 g, NDC<7 g and NDC, the sprouts number (NS) was also highly correlated to the corms number produced (0.61), since the daughter corms develop at the leaf base. As confirmed by ANCOVA, the MCW was positively correlated to all the production parameters (NF, NS, NDC and NDC≥7) and their R values were 0.35, 0.44, 0.48 and 0.48, respectively.

Table 6. Origins classification by the daughter corms number in saffron.

Original	Mean	Grouping
P8	**8.89**	**A**
P5	8.53	AB
P1	8.21	ABC
P6	7.61	BCD
P15	6.86	CDE
P9	6.65	CDE
P4	6.62	DE
P2	6.31	EF
P7	6.05	EF
P16	5.88	EF
P3	5.81	CDEF
P18	5.80	CDEF
P17	4.56	F

Values followed by a common letter within each column are not significantly different at P < 0.05 using the Duncan's Multiple Range Test (DMRT).

Table 7. Pearson correlation Matrix among agro-morphological traits in saffron.

Number of flowers (FN)	0.35***						
Number of shoots (NS)	0.44***	0.17***					
Plant heigth (PH)	0.15***	0.22***	0.38***				
Number of dauther corms (NDC)	0.48***	0.10**	0.61***	0.14***			
Number of dauther corms (NDC<7)	0.17***	-0.04^{NS}	0.40***	-0.12***	0.79***		
Number of dauther corms (NDC≥7)	0.48***	0.22***	0.35***	0.41***	0.35***	-0.29***	
-	MCW	FN	NS	PH	NDC	NDC<7	NDC≥7

*, **, ***: significant at 0.05, 0.01 and 0.001 respectively.

Table 8. Flowering percentage in Saffron over different origins.

ORI.	P1	P15	P16	P17	P18	P2	P3	P4	P5	P6	P7	P8	P9
FLOW (%)	**65.5**	31.0	20.3	18.8	33.3	21.8	31.3	20.7	28.3	25.8	41.7	41.2	42.9

Negative correlations were identified between NDC<7 and NDC≥7 (-0.29) and PH (-0.12) (Table 7).

Flowering potential and productivity

The first-year evaluation at Berrechid experimental station, showed that only 32% of the corms produced flowers. The flowering rate varied between origins and ranging from 1 to 3 flowers/corm (Table 8). P17 was the least productive population with less than one flower produced per 5 corms, while 65.5% of P1 corms exhibited flowers.

Saffron is known as a low volume and high value crop with the number of flowers per plant being the most determinant parameter for saffron yield, contributing directly to the production volume. Results revealed a wide variability for this trait ranging from 1 to 9 flowers produced per corm with an average of 2.2 flowers.

The stigma length and weight, two important yield contributing traits, recorded a wide range of variation for different regions, ranging between 32 and 38 mm and 4.2 and 6.2 mg, respectively. Maximum stigma weight (7.1 mg per flower) was recorded among accessions of P1 origin and the lighter DSW was recorded among P18 accessions.

Table 9. Descriptive analysis of saffron.

Ori.	N	MCW		FN		DA		FD		FED		FSL		DSW	
		AVG	CV	AVG	CV	AVG	CV	AVG	CV	AVG	CV	AVG	CV	AVG	CV
P1	38	24.00	20.4	3.1	63.4	58.7	6.5	3.2	79.5	60.9	5.5	**3.7**	10.1	**6.2**	17.6
P15	13	12.60	19.4	1.2	35.6	59.2	9.8	1.8	115.7	59.9	8.6	3.6	15.9	5.1	**25.8**
P16	13	16.20	13.9	2.2	45.4	57.6	4.9	**3.7**	74.5	60.3	3.5	**3.7**	11.4	5.4	24.8
P17	6	15.70	30.3	2	63.3	57.8	6.8	2.3	120.2	59.2	3.5	3.5	9.8	4.9	12.3
P18	5	15.10	32.5	1.4	39.1	61	4.5	1	0	61	4.5	3.2	14.4	4.2	19.1
P2	19	18.80	30.1	2.3	74.9	59.8	5.8	2.7	121.7	61.5	5.8	3.5	12.8	5.1	19.5
P3	5	10.20	34.3	1.2	37.3	61	**10.8**	1.8	99.4	61.8	**9.7**	3.7	12.1	5.6	12.9
P4	24	18.80	**42.9**	1.6	66.9	**61.5**	4.9	2	101.6	**62.4**	4.7	3.6	13.6	5.6	22.6
P5	36	25.60	32	2.3	62.6	58.8	4.9	2.9	83.7	60.7	4.7	3.5	**16.2**	4.7	20.6
P6	40	21.50	20	2.9	70.5	59.6	6.3	3.3	87.5	61.8	5.2	3.7	9.9	5.9	18.3
P7	25	21.70	29.4	2.3	**78.6**	60.2	8.3	2.8	**125.2**	62	7.7	3.6	14.3	5.8	18.9
P8	61	**26.40**	25.8	2.9	74.9	59.1	6.7	3.1	79.8	61.2	6	3.6	11	5.2	25.4
P9	21	19.60	32.2	**3.3**	55.6	58.8	7	**3.7**	79.2	61.5	6.8	**3.8**	8.1	5.6	18
-	-	18.94	27.94	2.21	59.08	59.47	6.71	2.64	*89.85*	61.09	**5.86**	3.59	12.28	5.33	19.68

MCW: Mother corm weight, FN: flowers number of flowered corms, DA: number of days to anthesis, FD: flowering duration, FED: number of days to 100% flowring. FSL: fresh stigma with style lenghtt, DSW: dray stigma with style weight, AVG: average, CV: variation coefficient, N: sample size, Ori: origin.

Table 10. Variance analysis of flowering traits.

Source	DF	FN	DA	FD	FED	FSL	DSW
MCW	1	39.9***	3.6	26.2***	2.6	0.0	9.7**
ORIGIN	12	2.6**	1.4	1.9*	1.0	1.8*	4.9***
Total	305	-	-	-	-	-	-

*, **, ***: significant at 0.05, 0.01 and 0.001 respectively.

The study confirmed the minimum variability for phenological and yield characters in saffron compared to agro-morphological traits (Table 9). Among different origins, the flowering duration period fluctuated between 15 and 21 days. The least variable trait was the number of days to the last flower emission (CV 5.98%). In contrast, flowering duration (CV 89.85%) as a phenological trait and the number of flowers (CV 59.08%) as a saffron production trait were the most variable.

Among agro-phenological traits, the MCW taken as a covariate had a very significant effect on the flowers number per corm (FN), the flowering duration (FD) and the stigmas weight (DSW) (Table 10). The differences between origins were on the other hand, very significant for the number of flowers per corm, the dry stigmas weight (DSW) and just significant for the flowering period duration (FD) and the fresh stigmas length (FSL). The differences between origins were not significant for the first and last days of flowering

Table 11. Pearson correlation matrix among phenological traits in saffron

Number of flowers (NF)	0.41***						
Day to anthesis (DA)	-0.11*	-0.45***					
flowering duration (FD)	0.29***	0.64***	-0.46***				
Number of days to 100% Flowering (FED)	0.10	-0.01	0.75***	0.24***			
fresh stigma length (FSL)	0.01	0.14*	-0.22***	0.12*	-0.16**		
Dry stigma weight (DSW)	0.13*	0.17**	0.03	0.08	0.09	0.46***	
-	MCW	NF	DA	FD	FED	FSL	DSW

*, **, ***: significant at 0.05, 0.01 and 0.001 respectively.

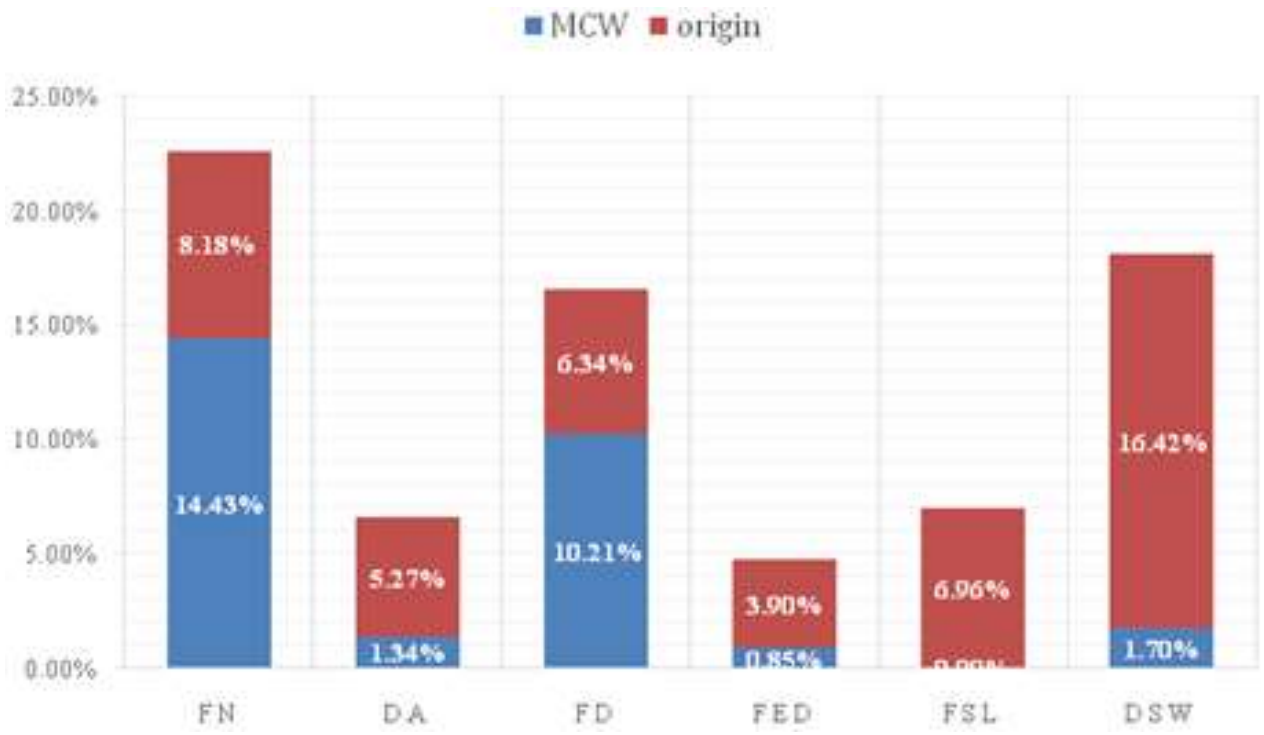

Figure 6. Contribution of the mother corm weight and the origin to total variance.

(DA, FED); this suggests that for the studied saffron collection, the phenological stages are mainly controlled by environmental factors.

The results showed that the contribution of mother corm weight to the total variance depends on the studied character. The highest variance rate explained by the mother corm weight was the flowers number (FN) (14.18%), followed by the flowering period duration (FD) (10.21%). High weight corms produce more flowers and therefore exhibit a longer flowering duration (Table 11). It is therefore concluded that the corm origin has a significant effect on saffron yield; this can be explained by the genotype profile and/or by the epigenetic effects of the provenances.

Soheilivand et al. (2007) reported differences in the flowers number produced by heavy and light corms and emphasized the fact that the heavier mother corms produce more flowers compared to the lighter corms. Planting corms weighing 3 to 7 g does not show any differences in the flowering rate or yield increasing. Mahdi et al. (2016) reported that corms with ≤6 g are not recommended for saffron cultivation.

The total variance due to origin was high for the fresh stigma length (FSL; 6.96%), dry stigma weight (DSW; 16.42%), and the flowering period duration (FED; 3.9%) (Figure 6). It is therefore concluded that the corm origin has a significant effect on saffron yield; this can be explained by the genotype profile and/or by the epigenetic effects of the provenances. Also, De-Mastro and Rota (1993) demonstrated that the corm size has a positive effect on the flowering rate but not on the stigma weight.

Table 12. Principal components for saffron populations.

PC	Variance (%)	Cumulated variance (%)
1	63.51	63.51
2	20.93	84.44
3	8.22	92.66
4	5.06	97.72
5	1.24	98.95
6	1.03	99.99
7	0.01	100.00

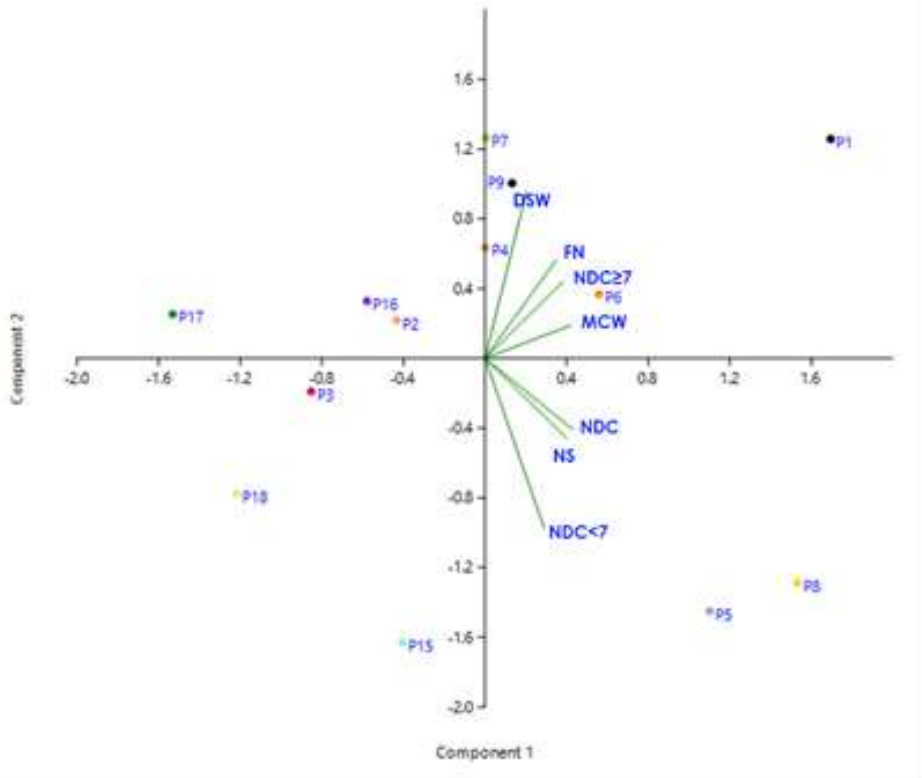

Figure 7. Principal Component biplot PC1 and PC2.

Principal component analysis

The principal components analysis revealed that the two first components had a cumulative variance of 84.44%, with the first PC cumulating 63.51% of the variance and the PC2 20.93% of the variance (Table 12).

The first component presented a positive strong loading from all the studied traits. The main contribution was from MCW, NS, NDC, NDC≥7 and FN, whereas it has a smaller contribution from NDC<7 and DSW. In contrary, the second component is mainly composed of a positive loading from DSW and a negative loading from NDC<7 (Figure 7).

The PCA biplot of the two first components have grouped studied populations into 5 homogeneous groups (Figure 8). The first group composed only of P1, as this population gathers individuals with a higher corm weight, NF, NDC≥7 and stigmas weight. The populations P5 and P8 form a second group and were projected on the positive side of PC1 and the negative side of PC2. The reason being a high MCW and the highest number of corms below 7 g associate with low stigma weight. Populations P17 and P18 were grouped together and are located on the negative side of the PC1 having a poor mother corms weight, number of flowers, shoot size and number, and number of daughter corms. P3 and P15

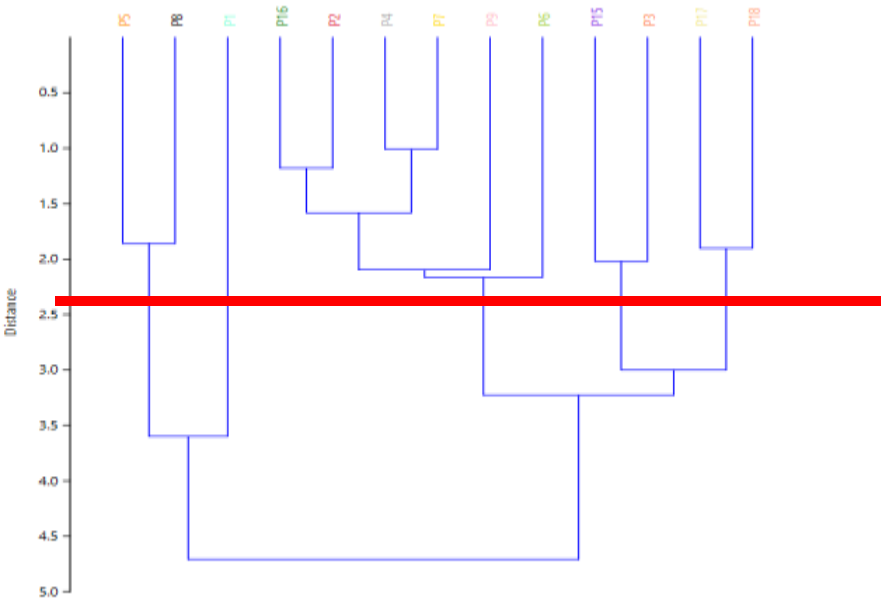

Figure 8. Populations clustering using UPGMA linkage and Euclidean distance.

populations belong to the fourth group located at the negative sides of both first and second principal components. This fourth group recorded the least mother corm weight, flowers number, number of daughter corms over 7 g and stigma weight. The remaining six populations, P2, P4, P6, P7, P9 and P16, were grouped into the fifth group in the middle of the biplot representing intermediate values of all the evaluated traits (Figure 7).

Cluster analysis did not reveal any clear relationship between diversity pattern and geographical origins (Different provenances). This can be explained by corm exchange between districts, villages and farmers (Birouk, 2009). The group III (P12, P2, p7, P9, P4, and P6) gathered several origins from different altitudes. The pattern of grouping indicated that geographical diversity was not an essential factor to group the genotypes from a particular source. Similar findings have been reported by Maqhdoomi et al. (2010) and Qadri et al., (2013).

Based on the results of the correlation coefficients, NF, NDC and NDC≥7 g traits are the most important traits to improve yield components and subsequently can increase the saffron yield. Saffron is indeed a perennial plant; its agronomic traits have high positive phenotypic and genotypic correlation with each other. Therefore, selecting an appropriate size quality saffron corm guaranteed a large NF and NDC≥7 g, and high DCW that will ensure high saffron yield in the first and the subsequent years. Mahdi et al. (2016) and Molina et al. (2005) reported that the limiting factor for the flowering is the small size of the corms. Soheilivand et al. (2007) reported that planting 3 to 7 g corms does not make any difference in the flowering rate or on the yield.

Conclusion

The present study which aims to show the genetic diversity of Moroccan saffron, has demonstrated a great variability of almost the studied traits. It could be assumed that the combination of more than one factor could explain the variation observed. The obtained results may bring valuable information to initiate saffron improvement program. Thus, selection of productive cultivar is possible and is the fastest way to improve saffron productivity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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