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Genotype, Planting Density and Seasonal Effects on Phenological Stages of Safflower (*Carthumustinctorious* L) in Sebele, Botswana

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Abstract: ***Field experiments were carried out in Botswana University of Agriculture and Natural Resources in Botswana (59^o 24'S, 95^o 25'E and 993 m above sea level) during winter and summer to evaluate the effect of genotype, plant density and growing season on phonological stages of safflower. The treatments were five safflower genotypes (Kiama, Gila-Pi 537 692, Sina-Pi 537 598, Pi 537 636 and Pi 527 710) and six plant densities (62, 500, 83, 333, 100, 000, 125, 000, 166, 666 and 200, 000 plants/ha).Increasing plant density resulted with decreased days to rossette, elongation, branching, flowering and physiological maturity even though there were inconsistencies at some planting densities. Safflower genotypes took 135-147 and 100-116 days after sowing (DAS) to reach maturity in winter and summer, respectively. This means, winter grown safflower took long days to reach physiological maturity compared to summer grown crops across all genotypes resulting with genotypes 'Gila' and 'Sina' as early and late maturing respectively, across seasons.***

Keywords: *Carthamus tinctorius L.*, Phenology, Genotypes, Plant density

1.Introduction

Safflower (*Carthamus tinctorius L.*) is a bushy, herbaceous, thistle like annual oilseed crop from composite family (Singh and Nimbkar, 2006 and Weiss, 2000). It is native to Asia, Mediterranean region and widely grown in arid and semi-arid regions (Labella et al., 2019 and El Latief *et al.*, 2012). Safflower is a multipurpose crop with uses ranging from a premium vegetable oil (Weiss, 2000 and Velasco and Fernandez-Martinez, 2001), medicinal/herbal uses (Li and Mündel, 1996 and Moloney, 1998), leafy vegetable (Singh and Nimbkar, 2006), animal feeding (Emongor and Oagile, 2017 and Singh and Nimbkar, 2006), food colouring, cut flower and textile dyes (Ekin, 2005).This crop is highly adaptive to most regions as its drought, saline and temperature tolerant (Emongor and Oagile *et al.*, 2017 andMoatshe et al., 2016).The research and cultivation of safflower has generated useful knowledge in most countries. This has contributed to most countries showing interest in safflower cultivation due to its diverse uses and adaptive potential. Cultivation of a new crop to a regional cropping system requires information on its performance under local agronomic and environmental conditions. Proper plant population densities with appropriate row and plant distance adjustment are the most essential factors for increased safflower grain yields (Moatshe *et al.*, 20167 and to Mazumdar *et al.*, 2007).Plant density among safflower cultivars has a significant influence on soil moisture availability, radiation distribution, photosynthetic activity (Mohamadzedeh, 2011).It also plays an important economic role, as seed price is an important part of the total production cost (Emongor et al., 2015). Therefore, information on the proper plant density for optimum production of safflower is necessary to design a management system that allows maximum expression of genetic potential required in Botswana. Phenology as the knowledge of crop progressive stages is essential to enhance proper timing of agronomic activities such as pest and disease control, nutritional management, irrigation regimes, prediction of crop yield (Bruns, 2009 and Shaykewich, 1995) and for assessment of genotypic adaptation to different geographical locations and environment (Martin et al., 1993). According to Bruns (2009), crops respond differently to environmental conditions at certain periods of growth to undergo transformation throughout their life cycle (Bruns, 2009). Every growth stage is referred as phenostage and signals a change in the crop development (Bruns, 2009and Loomis and Connor, 1998).For drought and climate change mitigation strategies, research on response of safflower genotypes towards phenology under Botswana condition is important for selection basing on relative probability of future terminal drought and to balance the risk of using genotypes with vegetative phase and flowering time that best suits their conditions. Since studies has been done on different crops including safflower and has revealed that there is a link between climatic variables, crop phenology and yield (Nguyen-Sy *et al.*, 2019 and LaBella *et al.*, 2019). It is reported that main factors affecting phenology includes temperature rise, changes in agronomic practices and variation in genotypes and varietal shifts (Nguyen-Sy *et al.*, 2019).Understanding crop phenology and adaptation is related with its inherent maturity traits (Mo et al., 2017 and Shimono *et al.*, 2009). Maturity determines patterns of crop development and its important determinant of crop yield on a specific location with climatic conditions (Shimino *et al.*, 2009). This means maturity or phenological stages of a crop to specific environments aids in climatic impacts on crop yields which can be useful during climate change mitigation strategies such as modelling (Kang and Tenhunen, 2009 and Nguyen-Sy *et al.*, 2019).It has been reported that late maturing cultivars are greater than early maturing cultivars. Late maturing cultivars have a longer reproductive period and can take full advantage of the water and thermal resources in growing season (Heverkort and Goudrian, 1994). The objective of the study is to determine the effect of

genotype, plant density and season on duration and/or timing of phenological stages of safflower.

2.Materials and Methods

2.1. Experimental site: Four field experiments were conducted in the Botswana University of Agriculture and Natural Resources content farm, located at latitude 59^{o} 24'S, 95^{o} 25'E and 993 m above sea level. Most rain falls in summer, which generally starts in late October and continues to March/April. The soils are shallow, ferruginous tropical soil, mainly consisting of medium to coarse grain sandy loams with a low water holding capacity and poor phosphorus values (De Wilt and Nachtengale, 1996). The mean rainfall is 538 mm per annum. The temperature variations are extreme throughout the year. In winter (mid-May until mid-August) temperature may range from -1^{o}C (morning) to 30°C (afternoon). In summer (mid- September to late mid-May) temperature ranges between 20^{o}C (morning) to 37^{o}C (afternoon) (Burgess, 2006).

2.2. Experimental Design: The experiment was laid in a randomised complete block design (RCBD) under split-plot arrangement with three replications. The treatments were five safflower genotypes and six plant densities evaluated under summer and winter growing seasons. The genotypes were Kiama composite (local), Sina-Pi 537 598, Gila -Pi 537 692, Pi 537 636 and Pi 527 710 randomly allocated to main-plots. Six plant population densities included 200, 000 (25 cm x 20 cm), 166, 666 (30 cm x 20 cm), 125, 000 (40 cm x 20 cm), 100, 000 (40 cm x 25 cm), 83, 333 (40 cm x 30 cm) and 62, 500 (40 cm x 40 cm) plants/ha randomly allocated to sub-plots. The sub-plots were 3 m x 3 m while the main-plots were 10 m x 7 m. The blocks were separated by 2 m space.

2.3. Cultural and Management Practices: The land was cleared, ploughed using mould board followed by disc harrowing to a fine soil tilth. Soil was sampled to determine mineral composition prior to planting. All necessary management practices such as pests, disease and weed control were undertaken to enhance good growth and development. The amount of water applied was according to crop water requirements (ETm) as related to reference evapotranspiration. The average water recommendation for safflower ranges between 600 - 1200 mm depending on climate and length of plant growth period (FAO, 2011).

2.4. Dependent variables determined: After sowing, different developmental stages were recorded to establish and categorise cultivars according to their time of maturity. The different phenological stages included days to 50% emergence, days from emergence to rosette stage, days to start of stem elongation, days to branching initiation, days to 50 % flowering, days to development of capitula, days to physiological maturity. Ten plants were tagged from each plot for determining the above phenological stages.

2.5. Statistical Analysis: Analysis of variance was performed on the data collected using general linear model (PROC GLM) procedure of Statistical Analysis System (SAS 2009, Carey, NC) program package.An appropriate regression model was used to examine the response of safflower germplasm to increasing planting densities. Multiple comparisons among means were done using Protected Least Significant Difference (LSD) at P = 0.05.Procunivariate procedure was carried out on residuals to support assumptions of normality made.

3.Results

There was no significant ($P < 0.05$) interaction of genotype and plant density on phenological performance of safflower, therefore only the main effects are described.

3.1. Effect of genotype on phenological stages of safflower

3.1.1. Emergence: Genotype had a significant ($P < 0.0001$) effect on emergence of safflower grown in winter and summer of 2015 and 2016, respectively (Table 1). However, during summer and winter of 2015 and 2016, respectively, safflower genotype did not have a significant effect on seed emergence (Table 1). During winter of 2015, the seeds of the genotype 'Kiama' took 10 days after sowing (DAS) to emerge which was significantly higher than that of the other genotypes which took on average 9 DAS to emerge (Table 1). The genotypes 'Gila', 'Sina', 'Pi 537 636' and 'Pi 527 710' did not significantly ($P < 0.05$) differ in their DAS to emergence in safflower grown during winter of 2015 (Table 1). In summer of 2015, the emergence of safflower seeds in all the genotypes ranged between 9-9.66 DAS (Table 1). Safflower grown during winter of 2016, emerged after an average of 9 DAS, but there was no significant ($P < 0.05$) genotype effect on emergence (Table 1). While safflower grown in summer of 2016 significantly ($P < 0.01$) differed in DAS to emerge (Table 1). The safflower seeds took 9-10 DAS to emerge in summer of 2016 (Table 1). The seeds of the genotypes 'Kiama', 'Gila' and 'Sina' took significantly ($P < 0.05$) more DAS than the seeds of the genotypes 'Pi 527 710' and 'Pi 537 636' (Table 1). However, the genotypes 'Kiama', 'Gila' and 'Sina' did not significantly ($P < 0.05$) differ in their DAS to seed emergence (Table 1). Similarly the seeds of genotypes 'Pi 527 710' and 'Pi 537 636' did not significantly ($P < 0.05$) differ in DAS to emergence (Table 1).

Growing season had a significant ($P < 0.0001$) effect on emergence of safflower seeds (Table 2). Safflower grown in summer took an average of 9.32 DAS to emerge which was significantly ($P < 0.05$) higher than 8.99 DAS taken by seeds grown in winter (Table 2). Also within seasons in both 2015 and 2016, there were significant differences ($P < 0.05$) with respect from DAS to safflower seed emergence (Table 1).

Table 1: Effect of genotype on phenological stages of safflower

Genotype	Emergence (DAS)		Rosette (DAS)		Elongation (DAS)		Branching (DAS)		Flowering (DAS)		Physiological maturity (DAS)	
Winter	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Gila	8.83b	8.67a	39.67b	35.33c	57.33a	49.67b	70.33ab	70.00a	111.61c	110.33c	135.00d	135.28d
Sina	8.89b	8.67a	40.22ab	40.67a	55.33b	56.00a	66.67c	66.67c	115.61a	116.67a	145.67a	146.67a
Pi 537 636	8.89b	9.00a	38.33c	40.00a	53.33c	54.61ab	66.67c	68.00b	113.33b	113.33bc	143.11b	143.67b
Pi 527 710	9.00b	9.00a	41.00a	41.00a	58.00a	59.33a	70.00b	70.33a	112.00c	112.89b	143.56b	144.67b
Kiama	10.00a	9.00a	37.33d	38.67b	55.00b	55.33a	71.33a	71.00a	115.00a	116.00a	139.06c	138.67c
Significance	****	NS	****	****	****	**	****	****	****	****	****	****
LSD	1.57	0.62	0.89	1.01	1.38	5.21	1.011	1.22	1.23	1.42	1.06	1.77
Summer	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Gila	9.00a	9.67a	30.67c	30.33d	44.33c	40.67c	60.00b	59.33c	90.67d	84.33b	100.00d	100.33a
Sina	9.66a	9.67a	35.00b	35.33bc	47.67b	47.67a	64.67a	67.33a	99.33a	87.00a	113.67b	102.33a
Pi 537 636	9.00a	9.00b	34.00b	34.33c	41.00d	40.00c	60.33b	59.33c	94.67b	81.67c	115.00a	100.67a
Pi 527 710	9.00a	9.00b	36.67a	37.33a	49.89a	48.33a	64.67a	65.33b	93.17cb	86.33a	115.67a	102.67a
Kiama	9.17a	10.00a	30.67c	36.00ab	51.33a	44.00b	60.67b	60.00c	92.00cd	84.00b	112.06c	100.00a
Significance	NS	**	****	****	****	****	****	****	****	****	****	NS
LSD	0.66	0.58	1.54	1.58	1.73	1.02	1.81	0.96	1.85	1.29	1.47	3.44

, **, NS Significance at P = 0.01, 0.0001 or not significant; Means separated using the Least Significant Difference (LSD) at P = 0.05, means with the same letter(s) within the column are not significantly different. DAS- days after sowing.

3.1.2. Rosette: There was a significant (P < 0.05) effect of genotype on number of days that safflower plants took to reach the rosette stage irrespective of year or season (Table 1). In winter of 2015, the genotype 'Pi 527 710' took 41 DAS to reach the rosette stage which was significantly (P < 0.05) longer than the time the genotypes 'Gila', 'Kiama' and 'Pi 537636' took to reach the rosette stage (Table 1).However, the genotype 'Pi 527 710' did not significantly (P < 0.05) differ in DAS to reach the rosette stage compared to the genotype 'Sina' (Table 1).Similarly, the DAS in which the genotype 'Sina' took to reach the rosette stage in winter of 2015 did not significantly (P < 0.05) differ to those of 'Gila' (Table 1). However, the genotype 'Sina' took 40.22 DAS to reach the rosette stage of winter 2015 which was significantly longer than the DAS that the genotypes 'Pi 537 636', 'Gila' and 'Kiama' took (Table 1). The genotype 'Kiama' took 37.33 DAS to reach the rosette stage in winter of 2015 which was significantly (P < 0.05) shorter than that of all the other genotypes (Table 1). In summer of 2015, genotype 'Pi 527 710' took 36.67 DAS to reach the rosette stage which was significantly (P < 0.05) longer than DAS other genotypes took (Table 1). While the genotypes 'Sina' and 'Pi 537 636' did not significantly (P < 0.05) differ in their DAS to reach the rosette stage in summer of 2015, but significantly (P < 0.05) took longer DAS to reach the rosette stage than the genotypes 'Gila' and 'Kiama' (Table 1). Both in winter and summer of 2015, the genotypes 'Pi 527 710' and 'Kiama' took the longest and shortest DAS to reach the rosette stage, respectively (Table 1).

In winter of 2016, the genotype 'Pi 527 710' took 41 DAS to reach the rosette stage which was significantly (P < 0.05) longer than DAS other genotypes took, with exception of the genotype 'Sina' and 'Pi 537 636' (Table 1). The response of genotypes 'Pi 527 710', 'Sina' and 'Pi 537 636' towards reaching rossette stage did not significantly (P < 0.05) differ (Table 1). The genotype 'Gila' took 35.33 DAS to reach the rosette stage which was significantly (P < 0.05) shorter than the DAS that all other genotypes took in winter of 2016.In summer of 2016, the safflower genotypes took in the range of 30.33-37.33 DAS to reach the rosette stage (Table 1). The genotype 'Pi 527 710' took 37.33 DAS to reach the rosette stage which was significantly (P < 0.05) longer than DAS which the genotypes 'Gila', 'Sina' and 'Pi 537 636' took, with exception of the genotype 'Kiama' (Table 1). The genotype 'Kiama' took 36 DAS to reach the rosette stage which was significantly (P < 0.05) longer than DAS which the genotypes 'Gila' and 'Pi 537 636' took, but was not significantly (P < 0.05) different from DAS which 'Sina' took (Table 1). The genotype 'Gila' took the shortest days (30.33 DAS) to reach the rosette stage compared to other genotypes planted in summer 2016 (Table 1).

The growing season significantly (P < 0.0001) influenced DAS in which the safflower genotypes reached the rosette stage (Table 2). In general safflower grown in summer reached the rosette stage 5.2 days earlier (34.03 DAS) than winter grown which was significantly (P < 0.05) different (Table 2). Within the growing season there was significant (P < 0.05) differences with respect to DAS in which the safflower plants took to reach the rosette stage (Table 1).

Table 2: Effect of growing season on phenological stages of safflower

Season	Emergence (DAS)	Rosette (DAS)	Elongation stage (DAS)	Branching stage (DAS)	Flowering stage (DAS)	Physiological maturity (DAS)
Winter	8.99b	39.23a	55.39a	69.10a	113.68a	141.53a
Summer	9.32a	34.03b	45.49b	62.17b	89.32b	106.24b
Significance	****	****	****	****	****	****
LSD	0.18	0.38	0.89	0.42	0.49	0.67

**** Significant at P = 0.0001, respectively. Means separated using the Least Significant Difference (LSD) at P = 0.05; Means with the same letter(s) within column are not significantly different. DAS- days after sowing

3.1.3. Elongation: Safflower genotype had a significant (P < 0.05) effect on DAS to when the plants reached the elongation stage (Table 1). During winter of 2015, the genotype 'Pi 527 710' reached the elongation stage after 58 DAS which was significantly longer than DAS of the genotypes 'Sina', 'Pi 537 636' and 'Kiama' with exception of the genotype 'Gila' (Table 1). The genotypes 'Sina', 'Kiama' did not significantly (P < 0.05) differ to reach the elongation stage (Table 1). The genotype 'Pi 537 636' took 53.33 DAS to reach the elongation stage which was significantly (P < 0.05) shorter than DAS of all other genotypes (Table 1). While in summer of 2015, genotype 'Kiama' took 51.33 DAS to reach the elongation stage which was significantly (P < 0.05) longer than DAS which all other genotypes took with exception of the genotype 'Pi 527 710' (Table 1). The genotype 'Pi 537 636' took 41 DAS to reach the elongation stage which was significantly (P < 0.05) shorter than DAS all the genotypes took (Table 1).

In winter of 2016, the genotypes 'Sina', 'Pi 537 636', 'Pi 527 710' and 'Kiama' did not significantly (P < 0.05) differ in their DAS to reach the elongation stage, but took significantly (P < 0.05) longer DAS than the genotype 'Gila' (Table 1). Also the genotypes 'Pi 537 636' and 'Gila' did not significantly (P < 0.05) differ in their DAS to reach the elongation stage in winter of 2016 (Table 1). In winter of 2016, the genotypes 'Pi 527 710' and 'Gila' took the longest (59.33) and shortest (49.67) DAS to reach the elongation stage, respectively (Table 1).During summer of 2016, the genotypes 'Pi 527 710' and 'Sina' did not significantly (P < 0.05) differ in their DAS to reach the elongation stage, but significantly (P < 0.05) took longer DAS than other genotypes (Table 1). The genotype 'Pi 537 636' took 40 DAS to reach the elongation stage which was significantly (P < 0.05) shorter than DAS of all other genotypes with exception of 'Gila' (Table 1). The genotype 'Kiama' took 44 DAS to reach the elongation stage in summer of 2016 which was significantly (P < 0.05) longer than DAS of the genotypes 'Gila' and 'Pi 537 636' (Table 1).

The growing season significantly (P < 0.0001) influenced DAS in which all the safflower genotypes reached the elongation stage (Table 2). There were significant (P < 0.05) differences within (Table 1) and between (Table 2) seasons with respect to DAS in which the safflower genotypes reached the elongation stage. Safflower grown in winter took on average 55.6 and 55 DAS in 2015 and 2016, respectively (Table 1). While in summer of 2015, safflower genotypes took on average 46.8 DAS to reach the elongation stage which was significantly (P < 0.05) longer than 44 DAS that plants took in summer of 2016 (Table 1). In general, safflower grown in winter significantly (P < 0.05) took longer DAS (55.39) to reach the elongation stage than summer grown safflower which took on average 45.49 DAS (Table 2).

3.1.4. Branching: There was a significant (P < 0.0001) effect of genotype on DAS that safflower plants took to reach branching stage irrespective of season or year (Table 1). Safflower plants grown during winter of 2015 took 66.67 to 71.33 DAS to reach the branching stage across genotypes (Table 3). The genotype 'Kiama' took 71.33 DAS to reach the branching stage which was significantly (P < 0.05) longer than DAS that the genotypes 'Sina', 'Pi 537 636' and 'Pi 527 710' took in winter of 2015 (Table 1). However, the time that 'Kiama' took to reach the branching stage did not significantly (P < 0.05) differ with that of 'Gila' in winter of 2015 (Table 1). The genotype 'Gila' took 70.33 DAS to reach the branching stage which was significantly (P < 0.05) longer than DAS that the genotypes 'Sina' and 'Pi 537 636' took in winter of 2015 (Table 1). However, the DAS of genotypes 'Gila' and 'Pi 527 710' were statistically similar (Table 1). Also the genotypes 'Pi 537 636' and 'Sina' did not significantly (P < 0.05) differ in DAS to reach the branching stage (Table 1).Safflower grown in summer of 2015 reached the branching after 60 to 64.67 DAS depending on genotype (Table 1). Safflower genotype significantly (P < 0.05) influenced the time plants reached the branching stage (Table 1). The genotypes 'Sina' and 'Pi 527 710' reached the branching stage at the same time (64.67 DAS), but took significantly (P < 0.05) longer DAS than the genotypes 'Gila', 'Pi 537 636' and 'Kiama' (Table 1). However, the genotypes 'Gila', 'Pi 537 636' and 'Kiama' did not significantly (P < 0.05) differ in their DAS to reach the branching stage (Table 1).

Safflower grown in winter of 2016 took 66.67 to 71 DAS to reach the branching stage depending on genotype (Table 1). The genotype 'Kiama' took 71 DAS to reach branching stage which was significantly (P < 0.05) longer than DAS that the genotypes 'Sina' and 'Pi 537 636' took (Table 1). However, the genotypes 'Kiama', 'Pi 527 710' and 'Gila' did not significantly (P < 0.05) differ in DAS to reach the branching stage (Table 1). The genotype 'Sina' took the shortest time (66.67 DAS) to reach the branching stage compared to all other genotypes (Table 1).Summer grown safflower in 2016 took 59.33 to 67.33 DAS to reach the branching stage depending on genotype (Table 1). The genotype 'Sina' took 67.33 DAS to reach the branching stage in summer of 2016 which was significantly longer than DAS all the other genotypes took (Table 1). The genotypes 'Gila', 'Pi 537 636' and 'Kiama' did not significantly (P < 0.05) differ in DAS to branching stage (Table 1). The genotype 'Pi 527 710' took 65.33 DAS to branching stage which was significantly (P < 0.05) longer than DAS that the genotypes 'Gila', 'Pi 537 636' and 'Kiama' took (Table 1). The genotypes 'Gila' and 'Pi 537 636' took 59.33 DAS which was the shortest duration to branching during summer of 2016 (Table 1). While the genotype 'Sina' took the longest DAS of 67.33 to reach the branching stage (Table 1).

Growing season had a significant (P < 0.0001) effect on branching of safflower (Table 2). Winter grown safflower on average took 69.1 DAS to reach the branching stage which was significantly (P < 0.05) longer than the duration summer (62.2 DAS) grown safflower took (Table 1).

3.1.5. Flowering: Genotype had a significant (P < 0.0001) influence on DAS to flowering of safflower whether grown in winter or summer (Table 1). In winter of 2015, safflower plants took 111.61 to 115.6 DAS to the flowering stage depending on genotype (Table 1). The

genotypes 'Gila' and 'Pi 527 710' took 111.61 and 112 DAS, respectively, to reach the flowering stage, which was significantly ($P < 0.05$) shorter than DAS that 'Sina', 'Pi 537 636' and 'Kiama' took in winter of 2015 (Table 1). The genotypes 'Sina' and 'Kiama' did not significantly ($P < 0.05$) differ in DAS to reaching the flowering stage (Table 1). Safflower grown in summer of 2015 took 90.67 to 99.33 DAS to reach the flowering stage depending on genotype (Table 1). The genotype 'Sina' took 99.33 DAS to reach the flowering stage in summer of 2015 which was significantly ($P < 0.05$) longer than DAS that all the other genotypes took (Table 1). While the genotype 'Gila' took 90.67 DAS to reach the flowering stage which was the shortest duration, and significantly ($P < 0.05$) shorter than DAS of the genotypes 'Sina', 'Pi 537 636' and 'Pi 527 710' (Table 1). The genotypes 'Gila' and 'Kiama' did not significantly ($P < 0.05$) differ in their DAS to flowering stage (Table 1).

Safflower grown in winter of 2016 took 110.33 to 116.67 DAS to reach the flowering stage depending on genotype (Table 1). The genotype 'Sina' took 116.67 DAS in winter of 2016 to reach the flowering stage which was significantly ($P < 0.05$) longer than DAS that other genotypes took with exception of 'Kiama' (Table 1). The genotype 'Gila' took 110.33 DAS to reach the flowering stage in winter of 2016 which was the shortest duration to flowering stage, and also significantly ($P < 0.05$) shorter than DAS of all other genotypes with exception of the genotype 'Pi 537 636' (Table 1). The genotypes 'Pi 537 636' and 'Pi 527 710' grown in winter of 2016 did not significantly ($P < 0.05$) differ DAS to reaching the flowering stage (Table 1). Safflower grown in summer of 2016 took 81.67 to 87 DAS to reach the flowering stage depending on genotype (Table 1). The genotype 'Sina' took 87 DAS to reach the flowering stage in summer of 2016, which was significantly ($P < 0.05$) longer than DAS other genotypes took with exception of 'Pi 527 710' (Table 1). The genotype 'Pi 537 636' took the shortest time (81.67 DAS) to reach the flowering stage in summer of 2016 and significantly ($P < 0.05$) shorter than the duration all other genotypes took (Table 1). The genotypes 'Gila' and 'Kiama' did not significantly ($P < 0.05$) differ in DAS to reaching the flowering stage in summer of 2016, but significantly ($P < 0.05$) longer than DAS which the genotype 'Pi 537 636' took (Table 1).

The growing season significantly ($P < 0.0001$) influenced DAS to flowering of safflower (Table 2). Winter grown safflower took (113.68 DAS) significantly ($P < 0.05$) 24.4 more DAS to reach the flowering stage than summer (89.32 DAS) grown safflower (Table 2). Within season the variation was small but significant (Table 3). Safflower plants grown in winter of 2016 significantly ($P < 0.05$) took 0.33 DAS (7.92 hours) to reach flowering stage compared to winter of 2015 (Table 3). While safflower grown in summer of 2015 (93.97 DAS) took 9.3 DAS longer than plants grown in summer (84.67) of 2016 (Table 3).

3.1.6. Physiological maturity: There was a significant ($P < 0.0001$) effect of genotype on physiological maturity of safflower except in summer 2016 (Table 1). The early and late maturing genotypes were 'Gila' and 'Sina', respectively, irrespective of season (Table 1). Safflower grown in winter of 2015 took 135 to 145.67 DAS to reach physiological maturity depending on genotype (Table 1). The genotype 'Sina' took 145.67 DAS to reach physiological maturity which was significantly ($P < 0.05$) longer than for other genotypes (Table 1). The genotype 'Gila' took significantly ($P < 0.05$) shorter time (135 DAS) to reach physiological maturity than all other genotypes grown in winter of 2015 (Table 1). The genotypes 'Pi 527 710' and 'Pi 537 636' did not significantly vary DAS to reach physiological maturity in winter of 2015 (Table 1). The genotype 'Kiama' in winter of 2015 took 139.06 DAS to reach physiological maturity which was significantly ($P <0.05$) shorter than DAS of genotypes 'Sina', 'Pi 537 636' and 'Pi 527 710' (Table 1).Safflower grown in summer of 2015 took 100 to 115.67 DAS to reach physiological maturity depending on genotype (Table 1). The genotype 'Pi 527 710' took 115.67 DAS to reach physiological maturity which was significantly ($P < 0.05$) longer than DAS which the genotypes 'Gila', 'Sina' and 'Kiama' took with exception of 'Pi 537 636' (Table 1). The genotype 'Gila' significantly ($P < 0.05$) took the shortest DAS (100) to reach physiological maturity compared to all other genotypes (Table 1).

Safflower grown in winter of 2016 took 135.28 to 146.67 DAS to reach physiological maturity depending on genotype (Table 1). The genotype 'Sina' took significantly ($P < 0.05$) the longest DAS (146.67) to reach physiological maturity compared to all other genotypes under study (Table 1). The genotype 'Gila' took significantly ($P < 0.05$) the shortest DAS (135.28) to reach physiological maturity compared to all other genotypes of safflower grown in winter of 2016 (Table 1). The genotypes 'Pi 537 636' and 'Pi 527 710' did not significantly ($P < 0.05$) differ in DAS to reach physiological maturity, but significantly ($P < 0.05$) took longer DAS than the genotypes 'Gila' and 'Kiama' (Table 1).In summer of 2016, safflower genotypes took 100 to 102.67 DAS to reach physiological maturity (Table 1). There was no significant ($P < 0.05$) genotype influence on DAS to reach physiological maturity in safflower grown in summer of 2016 (Table 1).

Growing season had a significant ($P < 0.0001$) effect on DAS to physiological maturity (Table 2). Safflower grown in winter took on average 141.53 DAS to reach physiological maturity which was significantly ($P < 0.05$) longer by 35.3 DAS than safflower grown in summer (Table 2). Within the season there were also significant ($P < 0.05$) differences (Table 3). Safflower grown in winter of 2016 took significantly ($P < 0.05$) 0.5 DAS to reach physiological maturity than safflower grown in winter of 2015 (Table 3). However, safflower grown in summer of 2015 took significantly ($P < 0.05$) 10 more DAS than safflower grown in summer of 2016 to reach physiological maturity (Table 3).

3.2. Effect of plant density on phenological stages of safflower

3.2.1. Emergence: Plant density had no significant

influence on days after sowing to emergence of safflower plants irrespective of season or year (Table 3).

3.2.2. Rosette: Safflower plant density had a significant ($P < 0.05$) effect on DAS to rosette stage in winter of 2015 (Table 3). In winter of 2015 safflower took 38.6 to 40 DAS to reach the rosette stage depending on plant density (Table 3). The response of safflower to reaching the rosette stage with increasing plant density was not consistent in winter of 2015 (Table 3). However, increasing safflower plant density from 62, 500 to 83, 333 plants/ha significantly ($P < 0.05$) delayed the plants to reach the rosette stage compared to plants at a density of 100, 000 and 125, 000 plants/ha in winter of 2015 (Table 3). In summer of 2015 plant density had no significant ($P < 0.05$) influence on DAS to the rosette stage (Table 4).

In both winter and summer of 2016, plant density had a significant ($P < 0.0001$) influence on DAS to the rosette stage (Table 4). In general increasing safflower plant density decreased the number of DAS to reach the rosette stage in both winter and summer of 2016 (Table 3). Safflower grown in 2016 took 38 to 41.18 and 33.53 to 36.73 DAS in winter and summer, respectively, to reach the rosette stage (Table 3). In winter of 2016 plants at density of 62, 500 plants/ha took 41.13 DAS to reach the rosette stage which was significantly ($P < 0.05$) longer than DAS that other plant densities took (Table 3). In summer of 2016, safflower plants at 62, 500 and 83, 333 plants/ha did not significantly ($P < 0.05$) vary in their DAS to the rosette stage, but were significantly ($P < 0.05$) longer than DAS of plant densities 125, 000 plants/ha and above (Table 3). Also plant densities of 100, 000 plants/ha and above did not significantly ($P < 0.05$) vary in DAS to the rosette stage in summer of 2016 (Table 3).

3.2.3. Elongation: There was no significant ($P < 0.05$) effect of plant density on DAS to the elongation stage of safflower with exception of summer 2015 (Table 4). In general, increasing safflower plant density reduced DAS to the elongation stage (Table 4). In general, safflower took 52.8 to 57.2 and 43.9 to 49.4 DAS in winter and summer, respectively, depending on plant density (Table 4). In summer of 2015, safflower plants at 62, 500 plants/ha took 49.4 DAS to reach the elongation stage which was significantly ($P < 0.05$) longer than DAS other plants in other densities took (Table 4).

3.2.4. Branching: Plant density had no significant ($P < 0.05$) effect on number of DAS to branching except in summer of 2015 (Table 3). In summer of 2015 safflower plant density significantly ($P < 0.05$) influenced DAS to branching (Table 3). In summer of 2015 increasing plant density significantly ($P < 0.05$) decreased DAS to start of branching (Table 3). Safflower plants at a density of 62, 500 plants/ha took 67.27 DAS to reach the branching stage which was significantly ($P < 0.05$) longer than DAS other plant densities took (Table 3). However, at 100, 000 plants/ha and above did not significantly ($P < 0.05$) differ in DAS to the branching stage in summer of 2015 (Table 3). Safflower plants at 100, 000 plants/ha and above took significantly ($P < 0.05$) shorter DAS to the branching stage than 83, 333 plants/ha (Table 3).

3.2.5. Flowering: There was a significant ($P < 0.05$) effect of safflower plant density on DAS to reach the flowering stage in winter of 2015 and 2016 (Table 3). However, plant density had no significant ($P < 0.05$) influence on DAS to flowering in safflower grown in summer of either 2015 or 2016 (Table 3). Generally safflower grown in winter took 112.53 to 115.60 DAS to flowering depending on plant density (Table 3). Safflower grown in winter at a density of 62, 500 plants took an average of 115.44 DAS to reach flowering which was significantly ($P < 0.05$) longer than DAS that other plant densities under study took (Table 3). Safflower plant density of 83, 333 plants/ha and above did not significantly ($P < 0.05$) vary in DAS to flowering in winter of either 2015 or 2016 (Table 3). In general, increasing plant density decreased DAS to flowering both in winter and summer grown safflower (Table 5).

In both winter and summer of 2016, plant density had a significant ($P < 0.05$) influence on DAS to the rosette stage (Table 3). In general increasing safflower plant density decreased the number of DAS to reach the rosette stage in both winter and summer of 2016 (Table 3). Safflower grown in 2016 took 38 to 41.18 and 33.53 to 36.73 DAS in winter and summer, respectively, to reach the rosette stage (Table 3). In winter of 2016 plants at density of 62, 500 plants/ha took 41.13 DAS to reach the rosette stage which was significantly ($P < 0.05$) longer than DAS that other plant densities took (Table 3). In summer of 2016, safflower plants at 62, 500 and 83, 333 plants/ha did not significantly ($P < 0.05$) vary in their DAS to the rosette stage, but were significantly ($P < 0.05$) longer than DAS of plant densities 125, 000 plants/ha and above (Table 3). Also plant densities of 100, 000 plants/ha and above did not significantly ($P < 0.05$) vary in DAS to the rosette stage in summer of 2016 (Table 3).

3.2.6. Physiological maturity: Plant density had significant ($P < 0.05$) influence on DAS to physiological maturity of safflower grown in winter of either 2015 or 2016 (Table 3). However, plant density had no significant ($P < 0.05$) influence on DAS to physiological maturity of safflower grown in summer of either 2015 or 2016 (Table 3). In winter of 2015 and 2016, plant density of 62, 500 plants/ha took 144 and 143.13 DAS, respectively, to physiological maturity which was significantly ($P < 0.05$) longer than DAS other plant densities took (Table 3). Plant densities of 83, 333 plants/ha and above did not significantly ($P < 0.05$) vary in DAS to physiological maturity in winter of either 2015 or 2016 (Table 3). In general, increase in plant density decreased DAS to physiological maturity of safflower (Table 3).

Table 3: Effect of plant density on phenological stages of safflower

Plant density	Emergence (DAS)		Rosette (DAS)		Elongation (DAS)		Branching (DAS)		Flowering(DAS)		Physiological Maturity (DAS)	
Winter	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
62, 500	9.20a	9.13a	39.73ab	41.13a	56.50a	57.20a	69.87a	68.60a	115.27a	115.60a	144.00a	143.13a
83, 333	9.20a	8.73a	40.00a	39.13b	56.47a	55.60a	68.40a	70.00a	113.20b	113.20b	140.27b	142.27b
100, 000	9.13a	8.80a	38.60c	38.73b	56.13a	55.60a	69.27a	69.93a	113.13b	112.87b	141.33b	142.13b
125, 000	9.07a	8.93a	38.87bc	38.13b	55.73a	55.27a	68.87a	69.47a	113.27b	113.53b	141.07b	141.67b
166, 666	9.00a	8.87a	39.07abc	38.73b	55.13a	55.47a	68.80a	68.53a	113.67b	114.00b	140.40b	141.54b
200, 000	9.13a	8.73a	39.60abc	38.00b	55.33a	52.80a	69.00a	68.67a	112.53b	113.87b	140.60b	141.43b
Significance	NS	NS	*	****	NS	NS	NS	NS	**	*	****	*
LSD	1.14	0.69	0.97	1.11	1.51	5.71	1.48	1.48	1.35	1.56	1.16	0.85
Summer	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
62, 500	9.33a	10.07a	33.80a	36.73a	49.40a	44.80a	67.27a	62.73a	94.50a	85.13a	144.00a	143.13a
83, 333	10.00a	9.73a	34.27a	35.80ab	46.07b	44.13a	63.87b	62.80a	93.87a	85.00a	140.27b	142.27b
100, 000	8.93a	9.27a	32.93a	34.27bc	46.73b	44.33a	61.60c	62.40a	93.80a	84.60a	141.33b	142.13b
125, 000	8.87a	9.20a	33.80a	33.87c	46.53b	44.27a	59.87c	61.93a	93.47a	84.50a	141.07b	141.67b
166, 666	9.07a	9.20a	33.20a	33.60c	46.33b	43.40a	59.67c	62.00a	92.67a	84.60a	140.40b	141.54b
200, 000	8.80a	9.33a	32.40a	33.53bc	46.00b	43.87a	59.13c	61.73a	92.47a	84.47a	140.60b	141.43b
Significance	NS	NS	NS	***	**	NS	****	NS	NS	NS	****	*
LSD	1.22	0.88	1.88	1.73	1.89	1.41	1.99	1.08	2.02	1.42	1.16	0.85

*, **, ***, ****, NS Significance at P = 0.05, 0.01, 0.001, 0.0001 or not-significant, respectively. Means separated using the Least Significant Difference (LSD) at P = 0.05; Means with the same letter(s) within column(s) are not significantly different. DAS- days after sowing

4.Discussion

Genotype and plant density had a significant influence on developmental stages of safflower in all the growing seasons (winter and summer). There was significant genotypic variation in all phenological variables (emergence, rosette stage, days to elongation, days to branching, days to anthesis and days to physiological maturity)investigated. The observation of genetic variation in phenological variables or traits in the current study is a valuable source for selection among safflower accessions and for breeding because the hybrids obtained from lines with more genetic variation shows more heterosis than convergent races (Golkar, 2012 and Golkar, 2014). In the current study, safflower genotypes under study took 135-147 and 100-116 days after sowing (DAS) to reach maturity in winter and summer, respectively. The early and late maturing genotypes were 'Gila' and 'Sina' both in winter and summer. Production of early maturing cultivars is a priority objective in many breeding programs. Plant earliness especially during flowering and maturity is an effective drought escape mechanism even though it can limit grain yield potential due to reduced time available for photosynthetic production and seed nutrient accumulation necessary for higher grain yield (Radhika and Thind, 2014).Shavroukov*et al* (2017) also reported that early flowering time and short vegetative phase is important under terminal drought conditions as it can minimise exposure of the crop to dehydration during the sensitive flowering and post-anthesis grain filling periods. Golkar (2011) reported that the rosette stage and days to bolling (end of elongation phase) were under the genetic control of additive effects. Some phenological traits such as days to flowering and physiological maturity are the most critical stages influencing safflower yield (Emongor et al., 2017; Golkar, 2014 and Weiss, 2000).Bruns (2009) also reported days to emergence and physiological maturity as the most contributing to plant earliness. The importance of both additive and dominance effects in the genetic control of earliness in safflower has been reported (Golkar, 2011 and Singh et al., 2008). The predominant role of additive gene action (Shahbazi and Seaidi, 2007) and the over dominance of gene action (Gupta and Singh, 1988) have also been reported to be important in the genetic control of days to maturity in safflower. Golkar (2011) also reported dominance gene effects involved in genetic control of days to flowering in safflower. While Gupta and Singh (1988) reported partial dominance effects on days to flowering in safflower. Golkar (2014) further explained that inconsistencies in genetic expression in safflower could be attributed to genetic and environmental interaction.

The growing season had a significant effect on all the phenological variables investigated in the current study. Safflower grown in winter had significantly longer phenological stages (rosette, elongation, branching, flowering and physiological maturity) than summer grown safflower. In winter and summer, the flowering and physiological maturity took up to 114 and 89 DAS, and 142 and 106 DAS, respectively, depending on genotype. The seasonal variation in the phenological variables observed in the current study was attributed to changes in temperature between winter and summer. During the experimentation period the average minimum and maximum temperatures were 4-16°C and 22-29^{o}C in winter. While in summer the average minimum and maximum temperatures were 17-23°C and 28-36^{o}C.Growing safflower in summer enhanced all the phenological stages of safflower due to the higher temperatures. The effect of growing season on safflower

phenological stages have been reported in literature (Emongor et al., 2015; Shabana *et al.*, 2013; Kedikanetswe, 2012; Ahadi et al., 2011; Golkar et al. 2011; Alizadeh, 2005 and Wachsmann *et al.*, 2001). Shabana *et al.* (2013) reported that safflower genotypes varied between 113-121 days from sowing to flowering. Wachsmann *et al.* (2001) stated that in Southern Australia, safflower took 31-43 DAS and 103-130 DAS for start of elongation and flowering phases, respectively, depending on safflower genotype. The length of the phenological stages was attributed to temperature and photoperiod during the growing period (Wachsmann *et al.*, 2001). Ahadi et al. (2011) reported that safflower phenological stages (days to flowering and maturity) were affected by sowing date in Iran and the phenological stages were prolonged with decrease in mean temperature. Golkar (2014) reported that days from sowing to emergence and/or maturity determines the early or late maturing genotypes of safflower. It has been reported that temperature affects all plants and their developmental stages from emergence to anthesis with greater impact experienced at the last phenological phases of crop life cycle (Slafer and Rawson, 1994). Goudriaan and Van-Laar, (1994) and Ritche and Ne Smith, (1991) emphasised that the rate of crop development is mainly temperature driven. In their findings, crop developmental rate was positively linearly correlated with temperature within an optimum range, with growth hastening as temperature increased (Goudriaan and Van-Laar, 1994).

Safflower plant density had a significant influence on phenological stages depending on the growing season and year in the current study. Increasing plant density from 62, 500 to 100, 000 plants/ha significantly decreased DAS to end of rosette, start of elongation, branching and flowering, and end of physiological maturity stages, depending on season and year in the current study. In winter increasing plant density from 62, 500 to 200, 000 plants/ha reduced DAS to flowering and physiological maturity by 1.73-2.74 and 1.7-3.6 depending on year. However, in summer grown safflower, plant density had no significant effect on DAS to flowering and physiological maturity. The reduction in DAS to end of rosette, start of elongation, branching and flowering, and physiological maturity was attributed to increased inter- and intra-competition for essential growth factors such as light, nutrients, water and photoassimilates. The findings of the current study are in agreement with those of Ahadi *et al* (2011) who reported decreased number of days of safflower from sowing to maturity as plant density increased from 20, 000 to 40, 000 plants/ha. Increasing safflower plant density from 300, 000 to 500, 000 plants/ha has been reported to enhance most of the developmental stages (Azari and Khajehpour 2003). Oad *et al.* (2002) reported that increasing safflower plant density from 74, 074 to 266, 667 plants/ha delayed crop maturity by 19 days (Oad *et al.* 2002). Dadashi (2004) and Varghar (2001) reported stress induced competition in safflower when plant density was increased above the threshold population resulting in reduced number of days from sowing to flowering. Increase in safflower plant density above threshold population results in increased intra-specific competition which limits the amount of above ground resources necessary for plant growth such as photoassimilates and quality sunlight, resulting in faster capitulum development and maturation for earlier completion of crop growth cycle (Sampaio et al., 2017 and Belle et al., 2012). Gan*et al* (2015) reported a significant decrease in number of days from 114 to 85 days to maturity as plant density increased from 200, 000 to 1, 000, 000 plants/ha. However, Amoghein et al. (2012a) reported that increasing safflower plant density from 300, 000 to 600, 000 plants/ha had no significant effect on days to emergence, end of rosette stage, start of branching, boll-bearing and flowering. Yadav (2014) in India reported that safflower genotype and plant density independently or via interaction had no significant effect on seed germination.

5.Conclusion

In Botswana safflower took 135-147 and 100-116 days after sowing (DAS) to reach maturity in winter and summer, respectively. This means winter grown safflower takes longer period to reach physiological maturity compared summer grown plants. From the study genotype 'Gila' and 'Sina' were identified as early and late maturing genotypes irrespective of season under Botswana conditions. In Botswana, genotype 'Gila' can be selected as an early maturing genotype this can be essential as a drought escape strategy to ensure active growth and metabolism for rapid completion of lifecycle before drought occurs while genotype 'Sina' is essential for selection of high yield as late maturing genotypes has more time for dry matter accumulation and grain filling process.

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