African Crop Science Journal, Vol. 28 Issue Supplement, s1 pp. 145 - 163 ISSN 1021-9730/2020 $4.00

DOI: https://dx.doi.org/10.4314/acsj.v28i1.11S

SAFFLOWER GENOTYPE BY PLANT DENSITY ON YIELD AND PHENOLOGICAL CHARACTERISTICS

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ABSTRACT

Safflower (*Carthamus tinctorius* L.) is a temperate plant grown in arid and semi-arid regions of the world, and is the most drought tolerant oilseed crop. The objective of this study was to evaluate the effect of genotype and plant density on growth, phenology and yield of safflower. Treatments included five safflower genotypes and six plant densities laid out in a randomised block design. Increasing plant density from 62,500 to 100,000 plants ha^{-1} significantly ($P < 0.05$) increased leaf area index (LAI), leaf area duration (LAD), total leaf chlorophyll content (Tchl) and net assimilation rate (NAR) at all phenological stages in both winter and summer. For all genotypes, the highest LAI, LAD, Tchl, NAR, total dry matter accumulation (TDM) and seed yield resulted at a plant density of 100,000 plants ha^{-1}. Maximum LAI, LAD, NAR and Tchl were observed at 50% flowering, compared to other phenological stages in all genotypes and plant densities. In general, genotype 'Sina' at 100,000 plants ha^{-1} significantly ($P < 0.05$) had the highest LAI, LAD, Tchl, TDM and seed yield compared to other genotypes and plant densities in both summer and winter.

Key Words: *Carthamus tinctorius*, leaf area duration, leaf area index

RÉSUMÉ

Le carthame (*Carthamus tinctorius* L.) est une plante tempérée cultivée dans les régions arides et semi-arides du monde. Il est la plante oléagineuse la plus tolérante à la sécheresse. L'objectif de cette étude était d'évaluer l'effet du génotype et de la densité végétale sur la croissance, la phénologie et le rendement du carthame. Les traitements comprenaient cinq génotypes de carthame et six densités de plantes disposées dans une conception en blocs randomisés. L'augmentation de la densité végétale de 62500 à 100000 plantes ha^{-1} de manière significative ($P < 0,05$) a augmenté l'indice de surface foliaire (LAI), la durée de la surface foliaire (DAL), la teneur totale en chlorophylle des feuilles (Tchl) et le taux net d'assimilation (NAR) à toutes les étapes phénologiques en hiver et en été. Pour tous les génotypes, les plus hauts LAI, LAD, Tchl, NAR, l'accumulation totale de matière sèche (TDM) et le rendement en graines ont abouti à une densité végétale de 100 000 plantes ha^{-1}. Un maximum de LAI, LAD, NAR et Tchl a été observé à 50% de floraison, par rapport à d'autres étapes phénologiques dans tous les

génotypes et densités végétales. En général, le génotype «Sina» à 100 000 plantes ha^{-1} ($P < 0,05$) avait le rendement en LAI, LAD, Tchl, TDM et en graines le plus élevé par rapport aux autres génotypes et densités végétales en été et en hiver.

Mots Clés: *Carthamus tinctorius*, durée de la surface foliaire, indice de la surface foliaire

INTRODUCTION

Safflower (*Carthumus tinctorious* L.) is drought and salt tolerant oilseed crop, from the family compositae (Singh and Nimbkar, 2006). The crop is multipurpose and has great nutritive and economic values for industrial, non-industrial, medicinal and food uses (Singh and Nimbkar, 2006; Emongor, 2010). Safflower is well adapted to the semi-arid and arid regions, where rain comes in winter and spring, and dry atmosphere prevails during maturation and flowering (Nikabadi *et al.,* 2008). Despite its adaptive abilities, genotype, plant density and other environmental factors (temperature, light, fertility and moisture) affect growth, physiology and yield (Sharifi *et al*., 2012). Sharifi *et al*. (2012) reported that the highest yield is generated when competition for growth factors reaches minimum level, and the plant is able to utilise environmental resources efficiently. As leaves emerge, their exposure to sunlight induces expression of genes coding for components of the photosynthetic apparatus (Foyer and Paul, 2001).

Plant leaf is a principal organ for photosynthetic activities, essential for light interception and dry matter accumulation (Mohankumar *et al*., 2005). Maximum leaf area results in more light interception, leading to high net canopy photosynthesis (Mohankumar *et al*., 2005). Monteith (1977) emphasised the extent in which the intercepted solar radiation by the plant canopy depends on the leaf area index (LAI) displayed both in space and time.

The potential traits for yield improvement include net assimilation rate (NAR), relative growth rate (RGR) and leaf area duration (LAD) (Ball *et al*., 2001). Net assimilation rate (NAR) as an indicator of photosynthetic efficiency (Gosh *et al*. 2013), reflects canopy development and its efficiency for radiation interception, thereby determining final crop yield (Stewart *et al*., 2013). Though LAI, LAD, NAR, CGR and RGR assist in understanding efficient plant type with potential of high yields, they are still affected by genotype and environmental factors. Therefore, understanding safflower crop growth, physiology and other related variables may be helpful in selection of superior genotypes for high yields to be used in breeding and/or crop production programmes under specific set of environmental conditions. The objective of this study was to evaluate the effects of genotype and plant density on crop growth, phenology and seed yield of safflower.

MATERIALS AND METHODS

Experimental site. Two field experiments (winter-April to September 2014 and summer-October 2015 to January 2016) were carried out in The Botswana University of Agriculture and Natural Resources (BUAN) Content Farm in winter and summer, located at latitude 59° 24'S, 95° 25'E and 993 m above sea level (De-Wilt and Nachtengale, 1996). Most rain falls in summer at an average mean of 538 mm per *annum*. Temperature during winter and summer may range from -1 °C (morning) to 30 °C (afternoon) and 20 °C (morning) to 37 °C (afternoon), respectively (Burgess, 2006). Soils mainly consisted of medium to coarse grain sandy loams and had low water holding capacity with poor phosphorus values (De-Wilt and Nachtengale, 1996).

Experimental design. The experiment was a split-plot laid in randomised complete blocks, with three replications. Treatments included five safflower genotypes (Kiama composite-local, Sina-Pi 537 598, Gila -Pi 537 692, Pi 537 636 and Pi 527 710), randomly allocated to main-plots and six plant population densities {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^{-1}}, randomly allocated to sub-plots. The sub-plots were 3 m x 3 m; while the main-plots were 10 m x 7 m.

Management practices. Before planting, the land was cleared, and disc ploughed and harrowed to a fine soil tilth. Safflower seed was sown directly with two seeds placed per hill, followed by thinning at 15 to 20 days after emergence. Management for pests, diseases and weeds were undertaken as necessary. The amount of water applied was according to crop water requirements (ETm), as related to reference evapotranspiration (FAO, 2011). The average water recommended for safflower growing ranges between 600-1200 mm, depending on climate and length of plant growth period (FAO, 2011). Irrigation was done once a week for 2 hours to achieve 11 mm, using over overhead sprinkler irrigation. Parameter measurements

Leaf area index (LAI). Ten leaves were sampled on ten plants per treatment replicate, to determine average leaf area (cm^2) at rossette (44 days after sowing (DAS)), branching (60-72 DAS), during 50% flowering (86-100 DAS) and post-flowering (100-120 DAS). This was done using the leaf area meter (Li-3100, Nebraska-USA). LAI was then calculated as the ratio of the total leaf area to unit ground area per plant.

Leaf area duration (LAD). This was estimated at rossette, branching, 50% flowering and post flowering using the formula below:

$$LAD = ((LAI_1 + LAI_2) \times (t_2 - t_1)) /2$$

Where:

LAI_1 and LAI_2 = the leaf area indices at 1st stage e.g. branch initiation (t_1) and 2nd stage e.g. onset of flowering (t_2), respectively (Hunt, 1978).

Total leaf chlorophyll (μgcm^{-2}). Total leaf chlorophyll content was determined using a portable chlorophyll meter (Minolta- SPAD-502 meter, Tokyo, Japan) from rossette to post-flowering stage. Mean chlorophyll content was determined from five leaves, sampled per plant and ten plants sampled per plot. Sampling was done on the mid-blade of randomly selected leaves (Uddling *et al.*, 2007). The chlorophyll values from the chlorophyll meter were converted into total chlorophyll content by multiplying the values by a factor of 0.84 (Uddling *et al.*, 2007).

Net assimilation rate (NAR). The average net assimilation rate was estimated at rossette, branching, 50% flowering and post-flowering (Hunt, 1978) using the following formula:

$$NAR\ (gm^{-2}day^{-1}) = TDM / LAD\ .$$

Where:

TDM = stand for total dry matter, and LAD = Leaf area duration.

Seed and total dry matter at harvest (biological yield). Seed yield was estimated from plants growing within a 4 m^2 area (central plot area). The seeds/grains were threshed and winnowed manually from the sample, and seed yield per unit area determined. The seeds were weighed using a digital weighing scale (Model 8800- Chicago, USA). Total dry matter (TDM) was determined by adding both seed and straw yield from unit ground area after air drying in the sun for 7 days.

Statistical analysis. Analysis of variance was performed on the data collected, using general linear model (PROC GLM) procedure of Statistical Analysis System (SAS) programme package. Multiple comparisons among means was done using Protected Least Significant Difference (LSD) at P>0.05.

RESULTS

Genotype by plant density on leaf area index (LAI). During winter, genotype and plant density had a significant ($P < 0.05$) interaction effect on leaf area index (LAI) from branching, 50% flowering and post-flowering stages (Figs. 1- 3). However, in summer, there was no significant ($P > 0.05$) interaction effect between safflower genotypes and plant density on LAI at different penological stages; therefore, only main effects are presented (Fig. 4). In general, LAI increased with increase in DAS, reaching a maximum at 50% flowering. Thereafter, LAI decreased irrespective of genotype, plant density or season (Figs. 1- 4). In winter, genotype 'Sina' had significantly ($P< 0.05$) higher LAI than all other genotypes planted in the different plant densities and phenological stages. In both seasons (winter and sumer), LAI increased with plant density, from 62,500 to 100,000 plants ha^{-1}, thereafter, it decreased at all phenological stages.

In winter, genotypes 'Gila' and 'Sina' at 62,000 and 100,000 plants ha^{-1} had the lowest and highest LAI in all phenological stages; with exception of post-flowering (Figs. 1- 3). During branching, in winter, genotype 'Sina' at 83,333 and 100,000 plants ha^{-1} did not significantly ($P > 0.05$) differ in LAI; however, LAI was significantly ($P < 0.05$) higher than in all the other genotypes planted in various plant densities (Fig. 1). Also, the LAI of the genotypes 'Kiama', 'Pi 537 636' and 'Sina' at 83,333, 100,000 and 125, 000 plants ha^{-1} did not significantly ($P > 0.05$) differ, at branching (Fig. 1). The maximum LAI value of 7.30 was attained at 50% flowering stage from genotype 'Sina' at 100,000 plants ha^{-1} (Fig. 2). At 50% flowering, LAI of winter grown genotypes 'Sina' and 'Pi 527 710' at 83,333 plants ha^{-1}, and 'Pi 537 636', 'Pi 527 710' at 100,000 plants ha^{-1} did not significantly ($P > 0.05$) differ, but was significantly ($P < 0.05$) greater than LAI of all other genotypes at all plant densities, expect 'Sina' at 100,000 plants ha^{-1} (Fig. 2). During this stage, genotype 'Gila' had the lowest LAI of 0.81 at 62,500 plants ha^{-1} (Fig. 2). At post-flowering in winter, LAI of genotype 'Sina' at 83,333 and 125,000 plants ha^{-1}, and 'Pi 527 710' at 83,333 and 100,000 plants ha^{-1}, respectively; did not significantly ($P > 0.05$) differ, but was significantly ($P < 0.05$) greater than LAI of all other genotypes

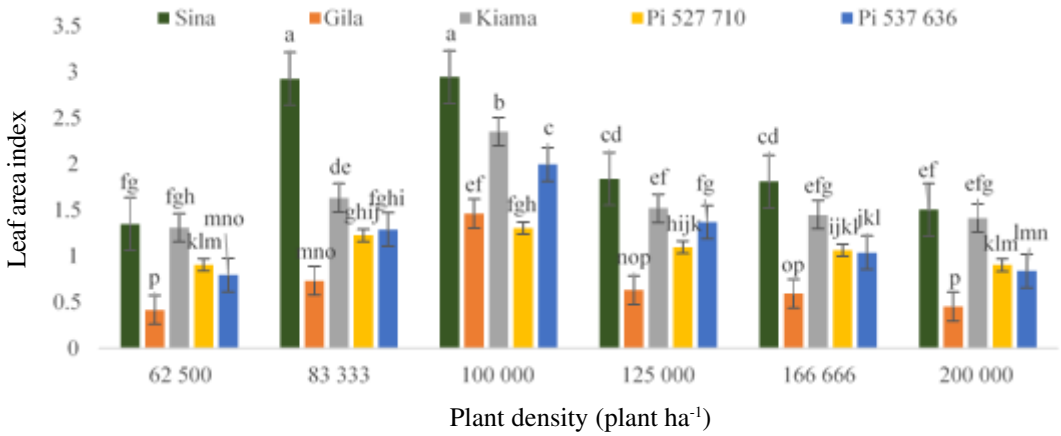

Figure 1. Effect of plant density and genotype on leaf area index of safflower in winter at branching stage; bars with the same letter are not significantly different,; mean separation by LSD at P=0.05.

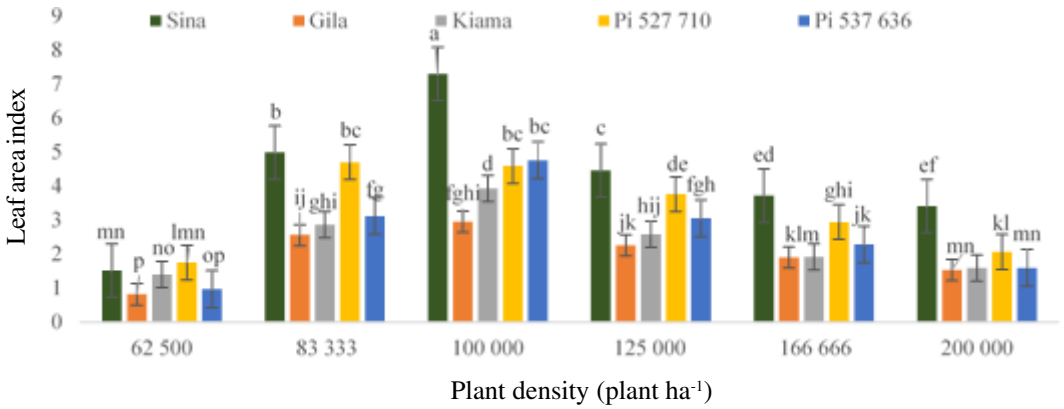

Figure 2. Effect of genotype and plant density on leaf area index of safflower in winter at flowering stage; bars with the same letter are not significantly different; mean separation by LSD at P=0.05.

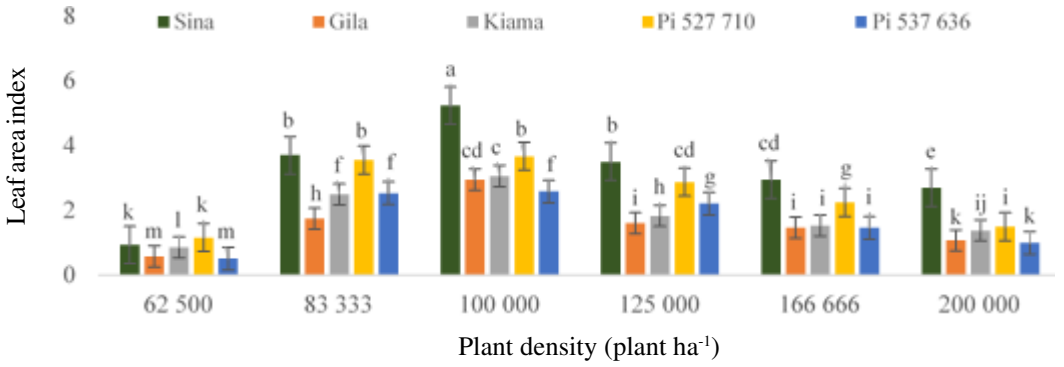

Figure 3. Effect of genotype and plant density on leaf area index of safflower in winter post-flowering; bars with the same letter are not significantly different; mean separation by LSD at P= 0.05.

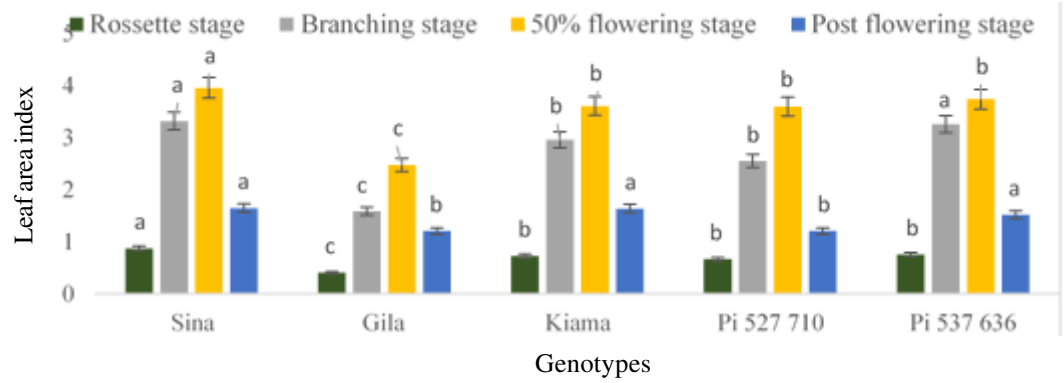

Figure 4. Effect of genotype on leaf area index of safflower at rossette, branching and flowering stage in summer; bars with same letter are not significantly different; mean separation by LSD at P = 0.05.

in different plant densities; with exception of 'Sina' at 100,000 plants ha^{-1} (Fig. 3). At this stage, LAI declined across genotypes resulting 'Pi 537 636' and 'Sina' recording 0.51 and 5.24 at 62, 500 and 100,000 plants ha^{-1}, had the lowest and highest LAI, respectively (Fig. 3).

Effect of genotype on LAI in summer. During summer, genotype 'Sina' had significantly ($P < 0.05$) higher LAI than other genotypes in all DAS (Fig. 4). In summer, at the rossette stage, LAI of the genotypes 'Kiama', 'Pi 527 710' and 'Pi 537 636' did not significantly ($P > 0.05$) differ, but were significantly ($P < 0.05$) higher than LAI of 'Gila', but lower than that of 'Sina' (Fig. 4). At this stage, LAI of 'Sina' was significantly ($P < 0.05$) higher than that of other genotypes (Fig. 4). During the branching phase, LAI of 'Sina' was significantly ($P < 0.05$) higher than the LAI of all other genotypes; however it did not significantly differ from that of 'Pi 537 636' (Fig. 4).

During flowering, LAI of genotypes 'Kiama', 'Pi 527 710' and 'Pi 537 636' did not significantly ($P > 0.05$) differ, but was greater LAI of 'Gila', thoughlower than that of 'Sina' (Fig. 4). Similar to winter grown plants, genotypes had the highest LAI during flowering compared to other stages of growth (Fig. 4). However, within genotypes, 'Sina' had significantly ($P < 0.05$) the highest LAI of 3.96, but 'Gila' had the lowest LAI of 2.48 (Fig. 5). Leaf area index began to decrease after flowering, irrespective of genotype (Fig. 4).

Plant density on LAI in summer. Plant density significantly ($P < 0.05$) influenced LAI of safflower grown in summer (Fig. 5). The minimum and LAI was attained at 62,500 plant densities and at maximum 100,000 plants ha^{-1}, in all stages of growth (Fig. 5). However, 50% flowering was the most outstanding with the highest LAI of 4.05, compared to other stages of phenology. At the rossette stage, the LAI of 83,333 and 100,000 plants ha^{-1} did not significantly ($P > 0.05$) differ, but was higher than the LAI of other plant densities. During branching, the LAI of safflower plants at 83,333, 125,000, 166,666 and 200,000 plants ha^{-1} did not significantly ($P > 0.05$) differ, but was lower than LAI of plants at 100,000 plants ha^{-1} (Fig. 5). At flowering stage, LAI of safflower planted at 62,500 and 200,000 plants ha^{-1} did not significantly ($P > 0.05$) differ, but was lower than LAI of plants at 83,333, 100,000, 125,000 and 166,666 plants ha^{-1} in summer.

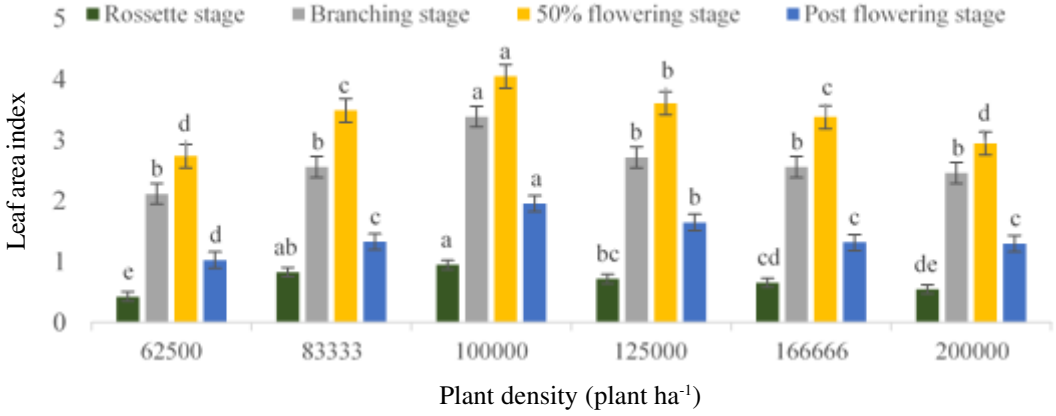

Figure 5. Effect of plant density on leaf area index of safflower at rossette, branching and flowering stage in summer; bars with same letter are not significantly different; mean separation by LSD at P = 0.05.

Genotype and plant density for leaf area duaration. Plant density and genotype had a significant ($P < 0.05$) interaction effect on leaf area duaration (LAD) of winter grown safflower (Figs. 6 and 7). This effect was not significant during summer; therefore, only main effects are described (Figs. 8 and 9). Similar to LAI, LAD increased with DAS, reaching a maximum at 50% flowering in both winter and summer grown safflower, across all genotypes and plant densities studied. Also, in both seasons, LAD significantly ($P < 0.05$) increased with increase in plant density up to 100,000 plants ha^{-1}; thereafter LAD decreased (Figs. 6 - 8).

During winter, genotype 'Sina' had significantly ($P < 0.05$) higher LAD values than other genotypes in all the developmental stages (Figs. 6 - 8). The maximum and minimum LAD values were attained by genotypes 'Sina' and 'Gila' at 100,000 and 62,500 plants ha^{-1}, respectively, at all phenological stages, with exception at branching stage (Figs. 6 - 8). At 50% flowering and at post-flowering stage, genotype 'Gila' had the lowest LAD value relative to all other genotypes and across all plant densities (Figs. 6 and 7). LAD of safflower at post-flowering stage declined across all genotypes (Fig. 8).

Plant density and LAD in summer. Plant density had a significant ($P < 0.05$) effect on LAD of safflower plants at different stages of phenology in summer (Fig. 8). Increasing in plant density from 62,500 to 100,000 plants ha^{-1} significantly ($P < 0.05$) increased LAD at

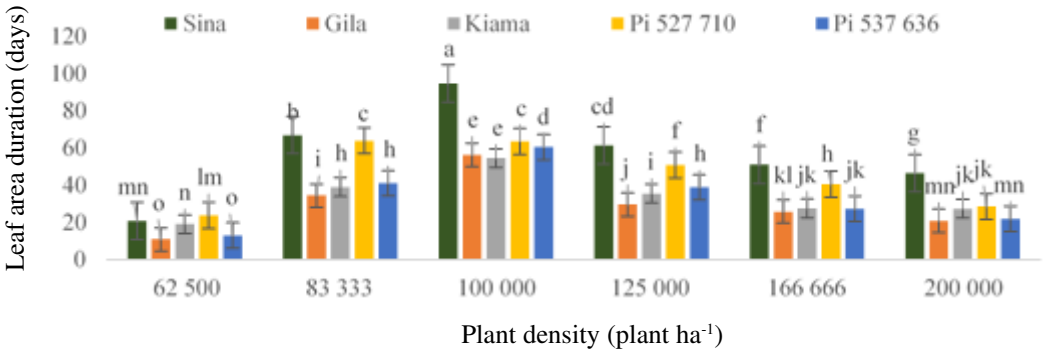

Figure 6. Effect of genotype and plant density on leaf area duration of safflower in winter at flowering stage; bars with the same letter are not significantly different; mean separation by LSD at P=0.05.

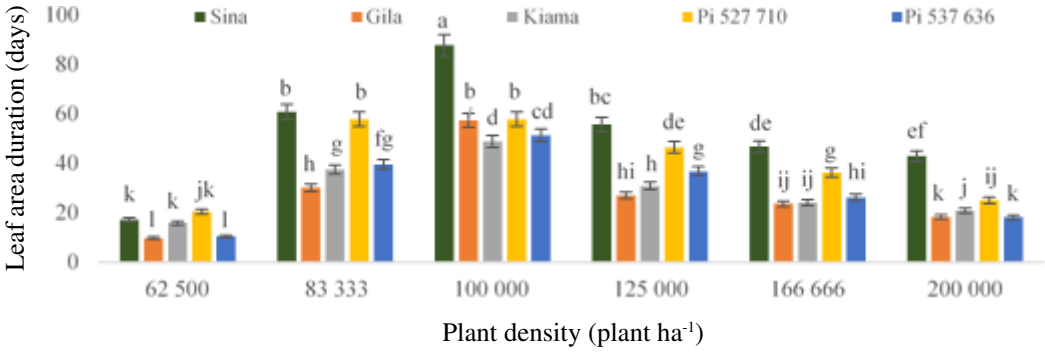

Figure 7. Effect of genotype and plant density on leaf area duration of safflower in winter, post-flowering; bars with the same letter are not significantly different; mean separation by LSD at P=0.05.

all stages of development (Fig. 8). LAD values significantly increased with increase in DAS, reaching a maximum at 50% flowering across all plant densities; thereafter, LAD decreased with increase in DAS post flowering (Fig. 8). The maximum LAD was 52.05 days at 100,000 plants ha^{-1} (Fig. 8). The percentage increase in LAD between rossette and branching stage in summer ranged between 26-42% (Fig. 8); while at 50% flowering stage in summer, LAD of safflower at 83,333, 125,000, 166,666 and 200,000 plants ha^{-1} did not significantly ($P > 0.05$) differ (Fig. 8).

Genotype and LAD in summer. There was a significant ($P < 0.0001$) effect of genotype on LAD of safflower grown in summer (Fig. 9). The LAD of summer grown safflower significantly ($P < 0.05$) increased between rossette to 50% flowering; thereafter, LAD decreased across all genotypes (Fig. 9). Maximum LAD was attained at 50% flowering (86 DAS) in all genotypes, but the highest LAD in summer was 48.98 days, in the genotype 'Pi 537 636'. The LAD values of all other genotypes were statistically similar at 50% flowering. In general, genotype 'Gila' had

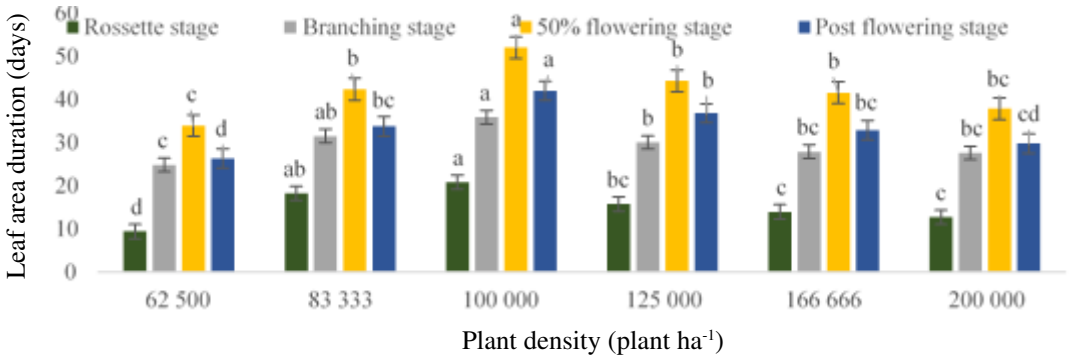

Figure 8. Effect of plant density on leaf area duration of safflower at rossette, branching and flowering stge in summer; bars with same letter are not significantly different; mean separation by LSD at P = 0.05.

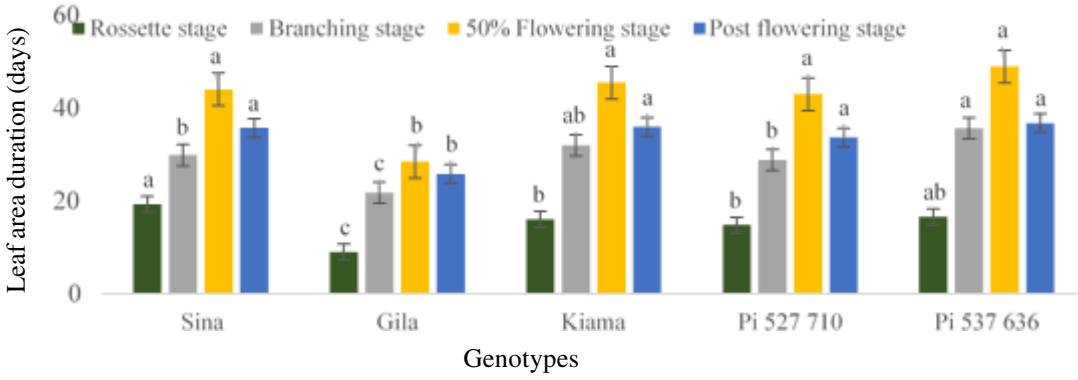

Figure 9. Effect of genotype on leaf area duration of safflower at rossette, branching and flowering stage in summer; bars with same letter are not significantly different; mean separation by LSD at P = 0.05.

significantly ($P < 0.05$) lower LAD than other genotypes in all stages of development (Fig. 9).

Genotype and plant density for net assimilation rate. There was a significant ($P < 0.0001$) interaction effect of genotype and plant density on net assimilation rate (NAR) of safflower grown in winter (Figs. 10 and 11). However, the results of summer grown plants did not show any significant ($P > 0.05$) interaction of genotypes and plant density on NAR of safflower; therefore only main effects shall be described (Figs. 12 and 13). The NAR

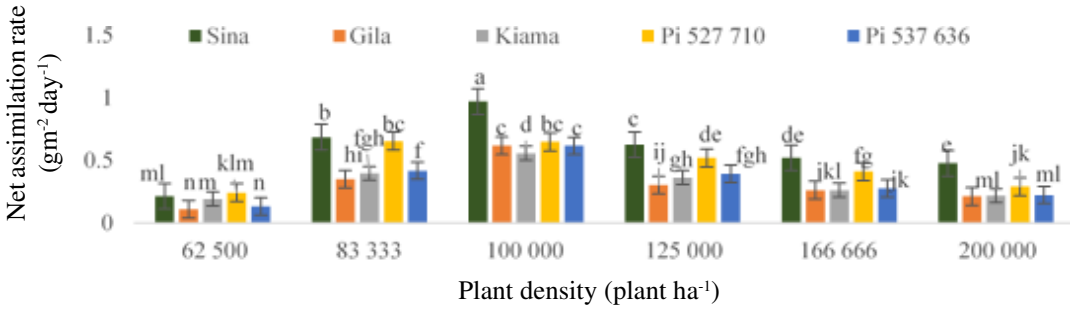

Figure 10. Effect of genotype and plant density on net assimilation rate of safflower in winter at 50% flowering; bars are not signifcantly different; mean separation bt LSD at P=0.05.

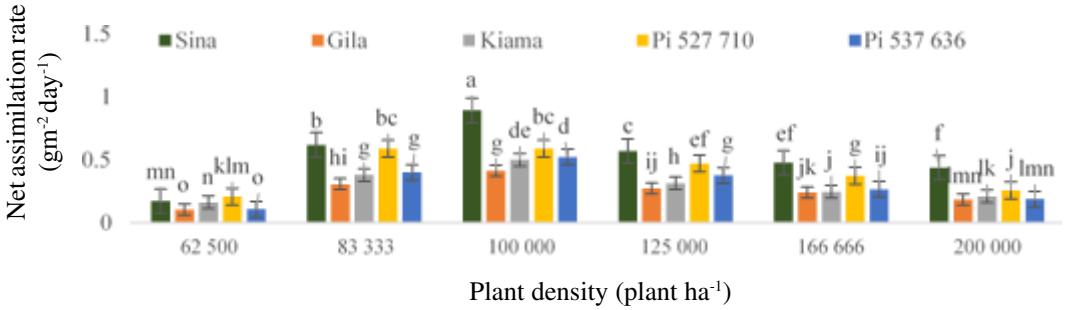

Figure 11. Effect of genotype and plant density on net assimilation rate of safflower in winter, post-flowering; bars with the same letter are not significantly different; mean separation by LSD at P=0.05.

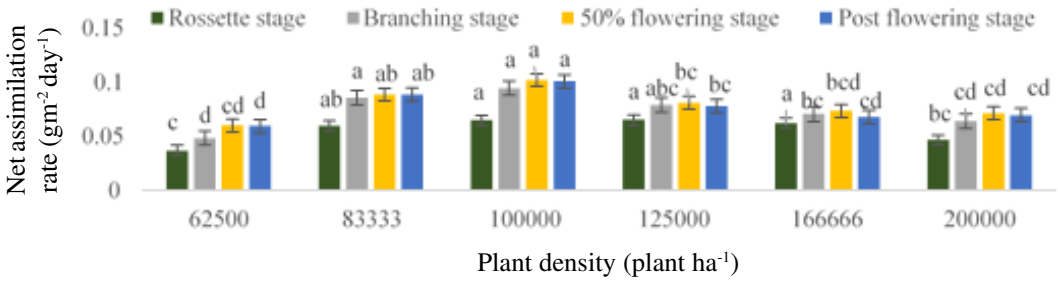

Figure 12. Effect of plant density on net assimilation rate of safflower at rossette, branching and flowering stage in summer; bars with same letter are not significantly different; mean separation by LSD at P = 0.05.

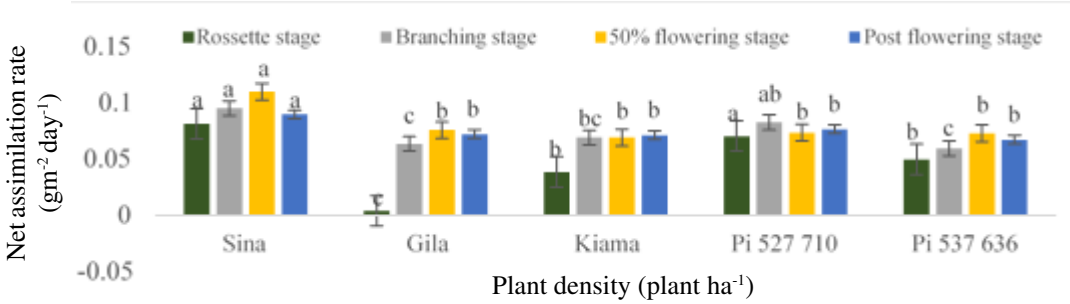

Figure 13. Effect of genotype on net assimilation rate of safflower at rossette, branching and flowering stage in summer; bars with same letter are not significantly different; mean separation by LSD at P=0.05.

significantly ($P < 0.05$) increased with increase in plant density up to 100,000 plants ha^{-1}. Thereafter, NAR decreased with increase in plant density in all genotypes in winter and summer (Figs. 10 and 11). During winter, genotype 'Sina' had higher NAR values in all plant densities compared to other genotypes (Figs. 10 and 11). Despite the non-significance of the interactions of safflower genotype and plant density experienced during summer, NAR significantly ($P < 0.05$) increased with increase in plant density reaching a maximum at 50% flowering; and thereafter NAR decreased (Figs. 12 and 13). Genotype 'Sina' had significantly ($P < 0.05$) higher NAR values than other genotypes, with maximum NAR attained at flowering (Figs. 12 and 13).

For winter grown plants, the minimum and maximum NAR values were attained by genotypes 'Gila' and 'Sina' at plant density 62,500 and 100,000 plants ha^{-1} at 50% flowering (0.89 gm^{-2}day^{-1}; 0.10 gm^{-2}day^{-1}) and post-flowering (0.89 gm^{-2} day^{-1}; 0.10 gm^{-2} day^{-1}) (Figs. 10 and 11). Safflower plants recorded the highest NAR at 50% flowering across genotypes at all plant densities (Fig. 10). The NAR of genotypes 'Sina' and 'Pi 527 710' at 83,333 and 100,000 plants ha^{-1}, respectively, was significantly ($P < 0.05$) higher than NAR of all other genotypes and in all plant densities at flowering; with exception of NAR of 'Sina' at 100,000 plants ha^{-1} (Fig. 10). The NAR of genotypes 'Sina' and 'Pi 527 710' at 83,333; 83,333 and 100,000 plants ha^{-1}, respectively, did not significantly ($P > 0.05$) vary, but were significantly higher ($P < 0.05$) than all other genotypes in all plant densities, expect 'Sina' at 100,000 plants ha^{-1} (Fig. 11).

Plant density and NAR in summer. There was a significant ($P < 0.01$) effect of plant density on NAR of summer grown safflower (Fig. 12). In summer, the maximum NAR was 0.102 gm^{-2} day^{-1} attained at flowering at plant density of 100,000 plants ha^{-1}; while the minimum NAR was 0.035 gm^{-2} day^{-1} at 62,500 plants ha^{-1}. There was dismal variation in NAR across phenological stages within a specific plant density. Thus, at 100,000 plants ha^{-1}, the NAR values did not significantly ($P > 0.05$) vary between stages of development (Fig. 12). In summer, NAR at all phenological stages did not significantly ($P > 0.05$) vary between 83,333 and 100,000 plants ha^{-1} (Fig. 12).

Genotype and NAR in summer. Genotype had a significant ($P < 0.0001$) effect on NAR of summer-grown safflower (Fig. 13). Genotype 'Sina' had significantly ($P < 0.05$) higher NAR than other genotypes in all phenological stages of summer grown safflower. Maximum NAR was 0.11 gm^{-2} day^{-1}, from genotype 'Sina' at flowering. The minimum NAR was 0.004 gm^{-2}day^{-1}, from genotype 'Gila' at rossette stage (Fig. 13). In summer, genotype 'Sina' had the highest NAR

in all phenological stages; however, it was not significantly ($P > 0.05$) different from NAR of genotype 'Pi 527 710' at rossette and branching stage. Also, the NAR of genotypes 'Gila' and 'Pi 537 636' during and after flowering, 'Kiama' in all phenological stages, and 'Pi 527 710' from branching and post flowering, respectively, did not significantly ($P > 0.05$) vary (Fig. 13).

Genotype and plant density for total chlorophyll content. Genotype and plant density had a significant ($P < 0.0001$) interaction effect on total chlorophyll content of safflower in winter (Figs. 14 and 15) and summer (Figs 16 and 17). In general, genotype 'Sina' planted at 100,000 plants ha^{-1} in both summer and winter significantly had ($P < 0.05$) higher total leaf chlorophyll content than any genotype planted at any density (Figs. 14 - 17). Similar to LAI, LAD and NAR, total leaf chlorophyll content significantly ($P < 0.05$) increased with increase in plant density from 62,500 to 100,000 plants ha^{-1}; thereafter, total

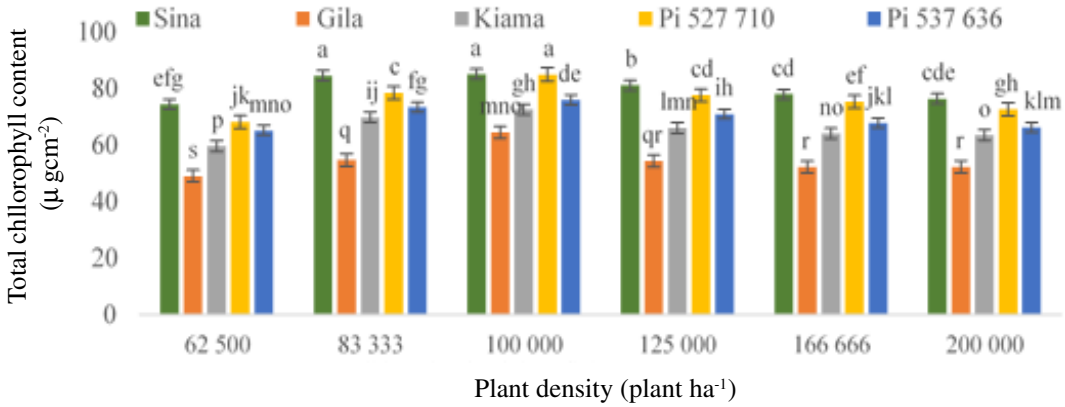

Figure 14. Effect of genotype and plant density on total chlorophyll content of safflower in winter at 50% flowering; bars with the same letter are not significantly different; mean separation by LSD at P=0.05.

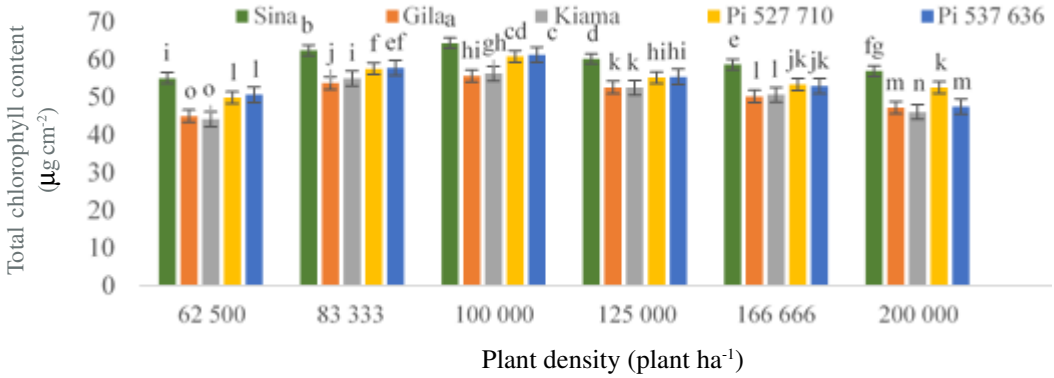

Figure 15. Effect of genotype and plant density on total chlorophyll content of safflower in winter, post-flowering; bars with the same letter are not significantly different; mean separation by LSD at P=0.05.

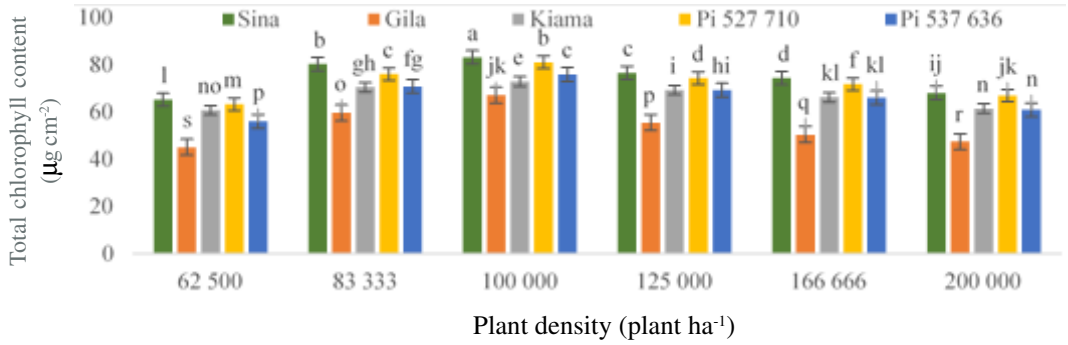

Figure 16. Effect of genotype and plant density on total chlorophyll content of safflower in summer at 50% flowering; bars with the same letter are not significantly different; mean separation by LSD at P=0.05.

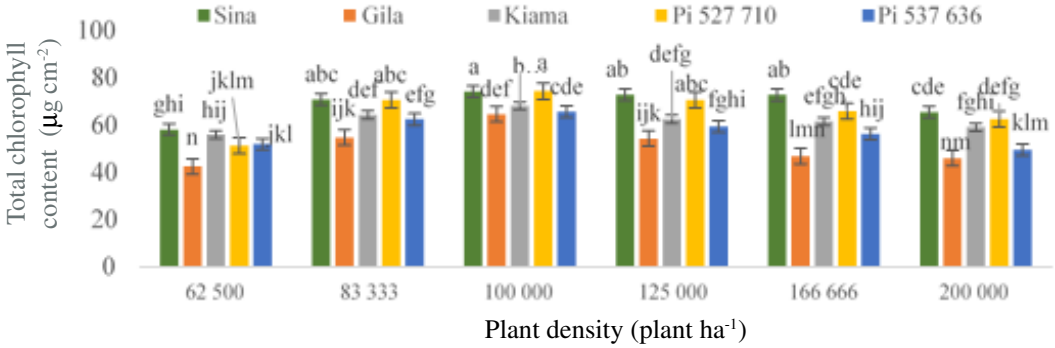

Figure 17. Effect of genotype and plant density on total chlorophyll content of safflower in summer, post flowering; bars with the same letter are not significantly different; mean separation by LSD at P=0.05.

leaf chlorophyll content decreased in all genotypes grown either winter or summer (Figs. 14 -17).

In winter, genotype 'Gila' at 62,500 plants ha^{-1} and 'Sina' at 100,000 plants ha^{-1} resulted in significantly ($P < 0.05$) the lowest and highest total chlorophyll content across phenological stages, except at post-flowering stage (Figs. 14 and 15). However, the maximum Tchl total chlorophyll content of 85.07 ìg cm^{-2} was observed during flowering stage from genotype 'Sina' at 100,000 plants ha^{-1} (Fig. 14). During flowering, the minimum (49.05 ìg cm^{-2}) and maximum (85.07 ìg cm^{-2}) total chlorophyll content was observed in genotypes 'Gila' and 'Sina' at 62,500 and 100,000 plants ha^{-1}, respectively, at 50% flowering (Fig. 14). However, genotype 'Sina' planted at 83,333 and 100,000 plants ha^{-1} did not significantly ($P > 0.05$) differ (Fig. 14). The total chlorophyll content of all genotypes and plant densities reached a maximum at 50% flowering, and thereafter, decreased post-flowering (Figs. 14 and 15). The minimum (44.17 ìg cm^{-2}) and maximum (64.33 ìg cm^{-2}) total chlorophyll content was observed in genotypes 'Kiama' and 'Sina' at 62,500 and 100,000 plants ha^{-1}, respectively, post-flowering (Fig. 15).

During summer, an increase in safflower plant density from 62,500 to 100,000 plants ha^{-1} resulted in an increase in leaf total

chlorophyll content (Figs. 16 and 17). At 50% flowering stage in summer, the leaf chlorophyll content of the genotype 'Sina' was significantly ($P < 0.05$) higher than that of all other genotypes planted in different plant densities (Fig. 16). Similar to winter-grown safflower at 50% flowering, the minimum (45.11 ìg cm^{-2}) and maximum (83.19 ìg cm^{-2}) total chlorophyll content was observed in genotypes 'Gila' and 'Sina at 62,500 and 100,000 plants ha^{-1}, respectively (Fig. 16). After flowering stage in summer, the total chlorophyll content of genotypes 'Sina' and 'Pi 527 710' at 83,333, 100,000 and 125,000 plants ha^{-1} was significantly ($P < 0.05$) higher than that of all other genotypes planted across plant densities with exception of 'Sina' at 166,666 plants ha^{-1} (Fig. 17).

Genotype and plant density for total dry matter. There was a significant ($P < 0.0001$) interaction effect between genotype and plant density on total drymatter (TDM) accumulation of safflower in winter and summer (Figs. 18 and 19). TDM signifcantly ($P < 0.05$) increased with increase in plant density from 62,500 to 100,000 plants ha^{-1}, then decreased as plant density increased

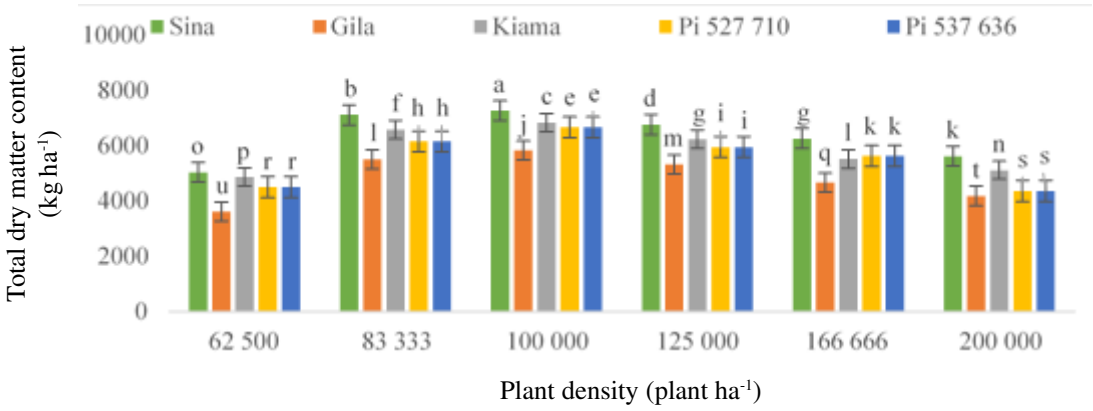

Figure 18. Effect of genotype and plant density on total dry matter content of safflower in winter; bars with the same letter are not significantly different; mean separation by LSD at P=0.05.

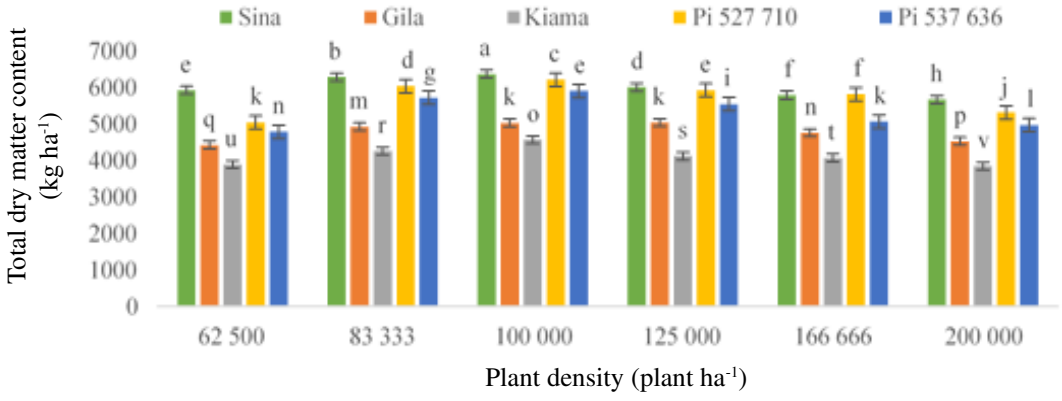

Figure 19. Effect of genotype and plant density on total dry matter content of safflower in winter; bars with the same letter are not significantly different; mean separation by LSD at P=0.05.

above 100, 000 plants ha^{-1} across all genotypes both in winter and summer (Figs. 18 and 19). The genotype 'Sina' at 100,000 plants ha^{-1} produced the highest dry matter accumulation of 7264 and 6371 kg ha^{-1} in winter and summer, respectively, which was significantly ($P < 0.05$) higher than that of other genotypes within and across densities (Figs. 18 and 19). Genotypes 'Gila' and 'Kiama' had significantly ($P < 0.05$) the lowest TDM of 3614 and 3895 kg ha^{-1} at 62,500 plants ha^{-1}, respectively, compared to all other genotypes and plant densities. In winter, safflower planted at 83,333 and 100,000 plants ha^{-1} significantly ($P < 0.05$) differed in their TDM, but was significantly ($P < 0.05$) higher than of any genotype and plant density (Fig. 18). Genotypes 'Pi 527 710' and 'Pi 537 636' planted at 100,000 plants ha^{-1} was similar, but significantly ($P < 0.05$) higher than TDM by other genotypes in various plant densities, with the exception of 'Sina' at 83,333, 100,000 and 125,000 plants ha^{-1}, and 'Kiama' at 100,000 plants ha^{-1}, in winter (Fig. 18). During summer, genotype 'Sina' planted at 83,333 and 100,000 plants ha^{-1} had significantly ($P < 0.05$) higher TDM than all genotypes across densities (Fig. 19). Genotype 'Pi 527 710' at 100,000 plants ha^{-1} had significantly ($P < 0.05$) higher TDM than all other genotypes planted in various densities, with the exception of 'Sina' at 83,333 and 100,000 plants ha^{-1} in summer (Fig. 19).

Genotype and plant density for seed yield. There was a significant ($P < 0.0001$) interaction effect between genotype and plant density as they influenced seed yield of safflower in winter and summer (Figs. 20 and 21). Seed yield significantly ($P < 0.05$) increased with

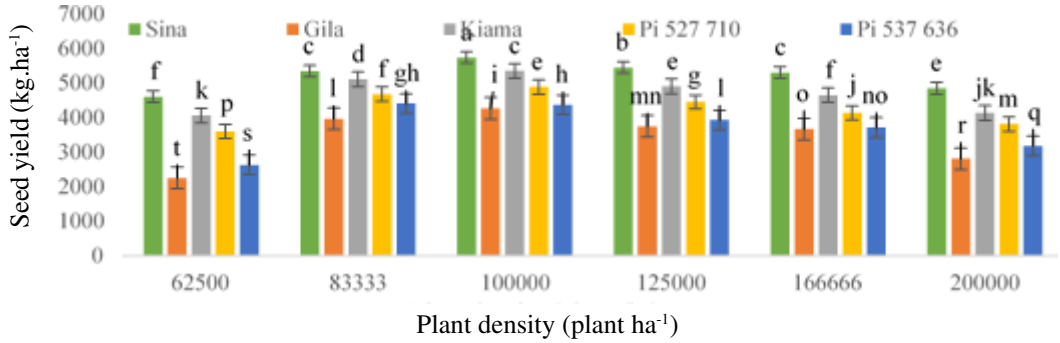

Figure 20. Effect of genotype and plant density on seed yield of safflower grown in winter; bars with the same letter are not significantly different; mean separation by LSD at P=0.05.

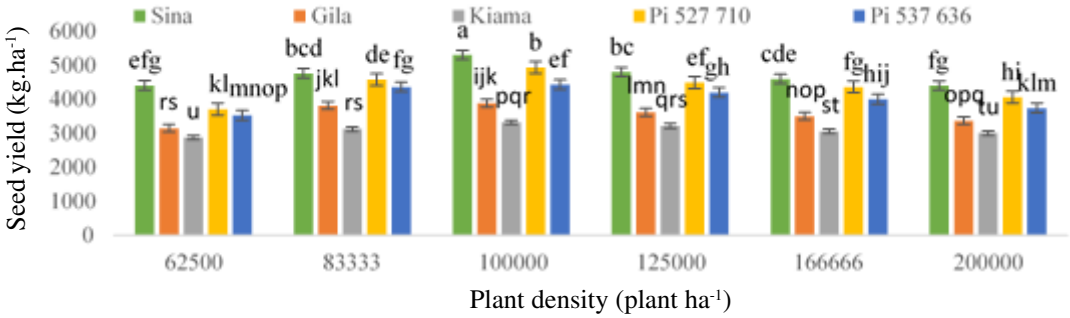

Figure 21. Effect of genotype and plant density on seed yield of safflower grown in summer; bars with the same letter are not significantly different; mean separation by LSD at P=0.05.

increase in plant density from 62,500 to 100,000 plants ha^{-1}, thereafter decreased across all genotypes both in winter and summer (Figs. 20 and 21). Genotype 'Sina' at density 100,000 plants ha^{-1} produced a seed yield of 5738 and 5300 kg ha^{-1} in winter and summer, respectively, which was significantly ($P < 0.05$) higher than the seed yield of other genotypes across densities (Figs. 20 and 21). At a plant density of 100,000 plants ha^{-1}, genotypes 'Gila' and 'Kiama' produced a seed yield of 4267 and 3324 kg ha^{-1} in winter and summer, respectively, which was significantly ($P < 0.05$) lower than the seed yield of other genotypes within the same plant density. Genotypes 'Gila' and 'Kiama' produced the lowest seed yield of 2251 and 2874 kg ha^{-1} in winter and summer, respectively, at a plant density of 62,500 plants ha^{-1}. During summer, seed yield of 'Sina' at 83,333 plants ha^{-1} was significantly ($P < 0.05$) higher than the seed yield of 'Kiama' at 100,000 plants ha^{-1} (Fig. 21). Seed yield of 'Sina' was stable across all plant densities (62,500-200,000) under investigation, ranging between 4609-5738 and 4409-5300 kg ha^{-1} in winter and summer, respectively (Figs. 20 and 21). In general, safflower plants yielded more seed in winter compared to summer across all plant densities and genotypes studied.

DISCUSSION

The significant interaction of genotype and plant density on LAI, LAD, Tchl and NAR observed in the present study is attributed to the response of genotypes to different plant densities through influencing safflower canopy development and efficiency for solar radiation interception, thereby influencing photosynthetic efficiency and ultimately yield. From the current study, all safflower genotypes had the highest LAI, LAD, total chlorophyll content and NAR at 50% flowering stage, irrespective of plant density or season. However, genotypes 'Sina' and 'Gila' planted at 100,000 and 62,500 plants ha^{-1}, respectively, had the highest and lowest LAI, LAD and NAR at anthesis, 100 and 86 DAS in winter and summer, respectively. Thus in comparison with other genotypes, a high LAI and Tchl was observed from the genotype 'Sina' at density 100,000 plants ha^{-1}, which implied efficient solar interception and photosynthesis. The high LAI and total chlorophyll content influenced a prolonged LAD of 'Sina' at density 100,000 plants ha^{-1}, which resulted high TDM and ultimately seed yield. The differences in genotypes with respect to LAI, LAD, total chlorophyll content and NAR at the same plant density was attributed to genetic factors. Safflower morphological traits such as LAI, LAD, total chlorophyll content, NAR and RGR have been reported to be influenced by additive and non-additive gene action (Shahbazi and Saeidi, 2007; Golkar, 2014). Genotypes 'Gila' and 'Sina' had the lowest and highest LAI, LAD, total chlorophyll content and NAR, respectively. Mokhtassi-Bidgoli *et al*., (2007) reported genotypic variation in leaf chlorophyll content, LAI and LAD in six safflower genotypes. The significant increase in LAI, LAD, NAR and total chlorophyll content with increase in safflower plant density from 62,500 to 100,000 plants ha^{-1} was attributed to adjustment of safflower plant canopy architecture. In most crops, plant density and spacing influence crop canopy architecture by modifying leaf size, leaf orientation and senescence of old and lower leaves (Tetio-Kagho and Garner, 1988; Ball *et al*., 2001). Sharifi *et al*. (2012) reported maximum photosynthetically active radiation (PAR) extinction coefficient (Kp) at low safflower plant densities, which resulted with increased LAI and LAD. The increase in NAR in the present study was attributed to the increase in LAI, LAD and leaf chlorophyll content as affected by both genotype and plant density during the vegetative growth.

The generally negative effect of plant density above 100,000 plants ha^{-1} on on LAI, LAD and total chlorophyll content may be attributed to inter-and intra-plant competition

for essential growth factors such as nutrients, sunlight and water. This observation is in agreement to those reported in literature (Sharifi *et al*., 2012; Vaghar *et al*., 2014; Emongor *et al*., 2015). From the current study, NAR followed the same trend and reduced at plant densities above 100,000 plants ha^{-1}. This was attributed to a decrease in LAI, LAD and total chlorophyll content. High planting densities above optimal range accelerated leaf senescence and increased competition for light due to excessive shading between leaves; while very low intra-plant densities below optimal range resulted in less light intercepted per plant. Low LAI makes the plant canopy inefficient in dry matter production. Omidi and Sharifmogadas (2010) reported that increasing safflower plant density above 200,000 plants ha^{-1} decreased NAR and crop growth rate (CGR), and there was a correlation between NAR and LAI. From the findings of the present study, the best safflower plant density irrespective of genotype under our growing conditions was 100,000 plants ha^{-1}.

In the present study, LAI, LAD, total chlorophyll content and NAR increased with increase in days after sowing (DAS), reaching a maximum at 100 and 86 DAS in winter and summer (flowering stage) grown safflower, respectively. Thereafter, all the crop growth variables decreased irrespective of genotype or plant density. The increase in NAR with DAS was attributed to the increase in LAI, LAD and total chlorophyll content. The significant decrease in LAI, LAD, NAR and total chlorophyll content after 100 and 86 DAS in winter and summer grown safflower was attributed to plant aging and leaf senescence. Safflower crop growth variables such as LAI, LAD, NAR and leaf chlorophyll content have been reported to increase during vegetative growth and reach a maximum during flowering (Mohamadi, 2006; Mokhassi-Bidgoli *et al.,* 2007; Hassan *et al*., 2015).

The increase in TDM and seed yield with increasing plant density up to a maximum of 100,000 plants ha^{-1} observed in the present study suggests increased photoassimilates as evidenced by the increase in LAI, LAD, NAR and total chlorophyll content. The highest TDM content of 7,264 and 6,371 kg ha^{-1}, and seed yield of 5,738 and 5,300 kg ha^{-1} was observed in winter and summer, respectively, at 100,000 plants ha^{-1}. Mirza *et al*. (2018) reported that increasing safflower plant density from 66,667 to 166,667 plants ha^{-1} significantly decreased plant dry matter. The highest dry matter was obtained at 66,667 plants ha^{-1} (Mirza *et al*., 2018). On the other hand, Hamza (2015) reported that increasing safflower plant density from 80,000 to 240,000 plants ha^{-1} significantly decreased the yield components. The variation in TDM and seed yield, as influenced by plant density in the present study compared with what is reported elsewhere may be due to differences in environmental factors such as moisture, season, temperature and soil differences (Sampaio *et al.,* 2017). Abaza (2010) reported a positive linear correlation between TDM with either LAI or LAD from emergence to maturity. The decrease in TDM of safflower in all genotypes with increase in plant density above 100,000 plants ha^{-1} in the present study may be attributed to the decrease in LAI, LAD, total chlorophyll content and NAR in the same plant densities due to intra- and inter-plant competition for essential growth factors.

In the present study, the highest safflower seed yield of 5738 and 5300 kg ha^{-1} in winter and summer, respectively, was produced by genotype 'Sina' at 100,000 plants ha^{-1} (40 cm x 25 cm). Seed yield observed in the present study was comparable to that reported by Moatshe *et al*., (2016) using safflower genotype 'Kiama' and 'Sina', respectively, where safflower planted at 100,000 plants ha^{-1} yielded 4248 and 4103 kg ha^{-1} of seed, respectively. The highest safflower yield of 'Sina' at density 100,000 plants ha^{-1} was attributed to increased LAI, LAD, total chlorophyll content, NAR and TDM in the

same plant densities from vegetative to flowering stage. Moatshe and Emongor (2019) reported that genotype 'Sina' was high yielding because it matured late, therefore, it had a longer LAD hence accumulated more TDM which was partitioned to grain filling. Dry matter accumulation is necessary for the translocation of photo-assimilates essential for flowering, pollination hence increased capitulum number per plant and grain filling resulting in increased seed yields (Sarkees and Tahir, 2016).

The superiority of most safflower parameters (LAI, LAD, total chlorophyll content, NAR, TDM and seed yield) in most phenological stages may be attributed to longer growth duration in winter caused by low winter temperatures (minimum and maximum temperatures were 4-16 and 22-29 °C), compared to higher temperatures in summer (minimum and maximum temperatures were 17-23 and 28-36 °C), the latter leading to faster growth. Similar observations have been reported in safflower, where accelerated increase in LAI and shorter LAD induced by high heat units in summer, thereby reaching senescence and aging earlier compared to winter grown safflower (Rasul, 2016).

ACKNOWLEDGEMENT

The authors are grateful to the Mastercard Foundation and Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) for funding research and publication cost through the Transforming African Agricultural Universities to meaningfully contribute to Africa's growth and development (TAGDev) Program.

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