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BREEDING INVESTIGATIONS INTO THE GENE ACTION AND AGRONOMIC PERFORMANCE OF SUNFLOWER TRAITS BASED ON F_1 TOP-CROSS HYBRIDS

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SUMMARY

Information on the performance and gene action of sunflower traits is still scarce in southern Africa despite the crop's increasing importance and the growing demand for adapted cultivars. To generate this information, 30 male-sterile lines were cross-bred to a restorer line generating 30 experimental hybrids. The hybrids and three check varieties were evaluated in South Africa, Zambia and Zimbabwe. Data was analysed using REML procedure in Gen-Stat®. Significant differences ($P<0.05$) were observed for grain yield traits, oil content and days to 50% flowering. Grain yield ranged between 1700 kg ha^{-1} and 4278 kg ha^{-1}, 1000-seeds weight between 59.4 g and 89.3 g, oil content between 36.6% and 44.6%, and days to 50% flowering between 55.5 and 68.0 for the hybrids but going up to 70 for the control varieties. Five hybrids were ranked above the highest performing check variety for grain yield. Two hybrids, HV9120 and HV9132, significantly outperformed the highest check variety by up to 19.6% and the lowest check variety by up to 91.6%. Oil content showed similar trends with eight hybrids having up to 6.8% yield advantage over the highest check variety and 19.3% over the lowest check variety. The high yields were attributed to heterosis and indicated the high potential for grain yield and oil content from the current germplasm set. The presence of high yielding hybrids with shorter days to 50% flowering, such as HV9132, HV9127, HV9128 and HV9135, showed that it was possible to breed for the short growing season, characteristics of most southern African areas, without compromising grain yield. General combining ability (GCA) effects were significant for all the traits indicating the importance of additive gene action for the traits. Parental lines KP1235, KP1304 and KP1290 that showed desirable GCA effects for at least three of the important traits, were selected for inclusion in the breeding programme for cultivars adapted to the region.

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INTRODUCTION

There is limited availability of sunflower (*Helianthus annuus* L.) cultivars adapted to the region of southern Africa. With the exception of South Africa, most countries in the region like Mozambique, Zambia, Malawi and Zimbabwe have weak to non-existent sunflower breeding programs. This is regardless of the high benefits derived from the crop such as confectionery, income from the sale of grain to oil producers and food for animals, and its additional use as a rotational crop. Lately, the call for the intensification of renewable energy production has seen sunflower transforming into an industrial crop because of its potential use as a biofuel crop. These uses can potentially improve rural economies if appropriate cultivars are available to the farmers, especially the small-scale farmers that are predominant in the region.

Sunflower has a high potential for grain yield and oil accumulation in the seed. Grain yield of between 1500 kg ha^{-1} and 4.5 kg ha^{-1} have been reported in the literature for both open-pollinated and hybrid cultivars (Arshad *et al.*, 2007; de Carvalho *et al.*, 2008; Ghaffari and Farrokhi, 2008; Sepehr *et al.*, 2008). Seed oil percentage of 38% to 54.4% and protein percentages of 15% to 17% have also been reported (Keshta *et al.*, 2008). However, these traits have been reported to be influenced by the germplasm and the environment in which they were evaluated (Hladni *et al.*, 2008; Rondanini *et al.*, 2003). This means that values recorded from elsewhere cannot be generally applied although they might be from the same germplasm set. This necessitates the need to evaluate germplasm in the cultivars' recommendation domain. Therefore, it is important to evaluate any materials to be used in a breeding program in the specific environment of deployment to gain an in-depth knowledge of the materials at hand.

The sunflower breeding program at the ARC-Grains Crops Institute houses many germplasm accessions, including cytoplasmic male-sterile (A) lines, their maintainers (B-lines) and fertility restorer (R*f*) lines, among other germplasm categories. Most of these lines have not been characterised and their performance in hybrid combinations for the important traits is not known. This information is important in selecting parents for inclusion in a hybrid development program. Selection of parents is vital and it requires an understanding of the genetics of the traits to be bred in the new cultivars. General combining ability (GCA) effects and specific combining ability (SCA) effects are used to determine the gene action involved in particular traits. The GCA effects are attributed to genes with additive effects while SCA effects are attributed to non-additive gene action (Sprague and Tatum, 1942). This information is available from other regions but scarcely reported for southern Africa. However, information obtained elsewhere cannot be generally applied to other regions because combining ability and inheritance information is specific to a germplasm set and environment (Falconer and Mackey, 1996). Therefore, there is need to generate this information from the current germplasm set evaluated in the region for the information to be applicable and meaningful to the breeding programme.

The chief selection criterion for parents in a hybrid oriented breeding program is their GCA effects, together with their means *per se*, SCA effects in hybrid combination and the hybrid means (Marilia *et al.*, 2001; Kashif and Khaliq, 2003). Rapid screening for GCA is usually done by crossing the inbred lines to a tester parent (Stuber, 1980). This is called top crossing and has been described and used by many authors including Jenkins (1978). A tester can be a low yielding variety (Allison and Curnow, 1966), a single cross (El-Ghawas, 1963), or an inbred line (Russell and Eberhart, 1975). Overall, the choice of tester depends on the simplicity of use and its screening power for GCA (Matzinger, 1953; Hallauer and Miranda, 1988; Menz *et al.,* 1999). The GCA is then assessed by comparing the average performance of a parent *inter se* to the overall performance of all the crosses in the crossing block. In sunflower, many authors have reported on the gene action involved in many traits, but using different sets of germplasm in different environments. Significant GCA effects have been reported for grain yield, days to maturity, plant height, weight of 1000 seeds, number of seeds per head, and oil content (Ghaffari *et al.*, 2011; Khan *et al.*, 2009; Khan *et al.*, 2008; Alza and Fernandez-Martinez, 1997). Studying gene action among inbred lines, Ortis *et al.* (2005), Kumar *et al.* (1998), Dagustu and Goksoy (2002), Shrikanth (1996) and Alba *et al.* (1985) reported additive gene action to be important for days to 50% flowering, seed yield and seed oil content. However, given the aforesaid environmental influence on the traits (Hladni *et al.*, 2008; Rondanini *et al.*, 2003) and the specificity of inheritance information to a set of germplasm and environment (Falconer and Mackay, 1996), the need to evaluate germplasm in the recommendation domain remains important in for cultivar development in any given breeding programme.

Taking the above mentioned into account, the objective of this study was to determine the general combining ability of A-lines and evaluate the performance of the resultant experimental hybrids across southern Africa. The information is valuable in a sunflower breeding program targeting the generality of the region of southern Africa.

MATERIALS AND METHODS

Experimental materials, sites and management

Thirty (30) cytoplasmic male-sterile lines, selected based on their performance in previous evaluations, with were top-crossed to a restorer (Rf) line, KP1326, to assess their general combining ability (GCA) as well as evaluate the agronomic potential of the resultant hybrids. Two crosses failed to yield enough quantities of seed. Therefore, the resultant 28 experimental hybrids and three commercial hybrid (CH) check varieties, CH1, CH2 and CH3, were evaluated in replicated α-lattice designs in South Africa at Potchefstroom, Zimbabwe in Harare and Zambia at Mt. Makulu Research Stations during 2008/2009 rainy season. The trials were laid out as α-lattice designs replicated three times per site. The trials were supplied with 200 kg ha^{-1} basal (3:2:1, N:P:K) and 200 kg ha^{-1} (28% N) top dressing fertilisers. Standard agronomic practices were followed and supplementary irrigation was supplied where necessary to achieve. The fields were kept weed free through hand weeding and harvesting was done by hand at harvestable maturity.

Data collection and analyses

Data was collected on grain yield, weight of 1000 seed, oil content (%) and days to 50% flowering. Grain yield data was collected separately from each plot at physiological maturity. Seed was left to naturally dry until no further loss was recorded over a period of two weeks. Yield was converted from a per-plot to a per-hectare and yield plant basis. Weight of 1000 seeds was measured by counting 100 seeds, weighing with a digital balance in the laboratory and multiplying the result by 10. The percentage oil content was measured using a Newport Analyser Type Watson 10 (Oxford Instruments, Newport Pagnell, England) and days to 50% flowering were assessed as the date on which 50% of the plants in a plot had flowered. All entry data (including checks) analyses were performed by REML procedure using a fixed effects model in GenStat® (Payne *et al.*, 2007) as follows:

$Y_{ijkl} = \mu + s_i + r_j(s_i) + b(r_j{*}s_i) + v_k + s{*}v_{ik} + e_{ijkl}$

where: Y_{ijk} =observed entry response; μ =overall population mean; s_i =effect of the i^{th} environment; $r_j(s_i)$ =effects of the j^{th} replication in the i^{th} environment; $b(r_j{*}s_i)$ =effects of the i^{th} block in the j^{th} replication in the i^{th} environments; v_k = effect of the k^{th} entry; $s{*}v_{kl}$ =interaction effect of the i^{th} environments and the k^{th} entry; and e_{ijkl} is the experimental error.

Combining ability analyses for the female parents were also performed by REML procedure using a fixed effects model in GenStat® (Payne *et al.*, 2007) as follows:

$Y_{ijkl} = \mu + s_i + r_j(s_i) + b(r_j{*}s_i) + f_k + s{*}f_{ik} + e_{ijkl}$

where: Y_{ijk} =observed entry response; μ =overall population mean; s_i =effect of the i^{th} environment; $r_j(s_i)$ =effects of the j^{th} replication in the i^{th} environment; $b(r_j{*}s_i)$ =effects of the i^{th} block in the j^{th} replication in the i^{th} environments; f_k = effect of the k^{th} female parent; $s{*}f_{kl}$ =interaction effect of the i^{th} environments and the k^{th} female parent; and e_{ijkl} is the experimental error.

The GCA effects for the female parents were calculated according to Kearsey and Pooni (1996) while their standard error (SE) and standard error of a difference (SED) were calculated according to Dabholkar (1992). Yield advantage over the check varieties (%) was computed over (i) the mean of the highest performing check

Table 1: Traits of sunflower hybrids evaluated in South Africa, Zambia and Zimbabwe during the 2009/10 rainy season

Entry	Grain yield		1000 seed weight (g)	Oil %	Days to 50% flowering	Plant stand	Grain yield (kg ha^{-1}) standard heterosis (%) relative to:		
	kg ha^{-1}	g plant^{-1}					Highest check	Lowest check	Mean of checks
HV 9120	4278	88.3	83.5	40.5	67.0	22.50	119.6	191.6	151.2
HV 9132	4097	70.7	75.8	43.1	58.5	26.30	114.5	183.5	138.1
HV 9141	3706	76.9	-	44.0	60.3	22.50	103.6	166.0	131.0
HV 9115	3661	67.0	71.6	40.3	66.4	24.20	102.3	163.9	129.4
HV 9111	3617	71.6	75.5	41.3	66.8	20.30	101.1	162.0	127.8
CH3[a]	3578	89.4	89.3	41.8	63.8	18.50	100.0	160.2	126.4
HV 9109	3578	62.5	69.9	36.6	67.6	19.60	100.0	160.2	126.4
HV 9127	3528	74.4	79.5	42.6	58.3	22.50	98.6	158.0	124.7
HV 9121	3506	66.8	70.3	40.3	67.9	21.20	98.0	157.0	123.9
HV 9129	3506	80.1	74.3	40.3	65.1	18.50	98.0	157.0	123.9
HV 9128	3483	79.4	86.8	42.5	57.8	21.00	97.3	156.0	123.1
HV 9140	3419	79.5	66.6	39.8	62.9	21.80	95.6	153.1	120.8
HV 9131	3411	72.3	74.8	40.1	65.1	19.50	95.3	152.8	120.5
HV 9125	3306	65.1	81.0	44.6	60.8	23.00	92.4	148.1	116.8
HV 9135	3172	83.0	73.8	43.1	58.3	17.30	88.7	142.1	112.1
HV 9124	3167	73.0	76.0	40.3	68.0	17.60	88.5	141.8	111.9
HV 9136	3165	124.2	73.7	40.4	63.7	18.60	88.5	141.7	111.9
HV 9117	2883	63.5	80.5	40.8	57.0	20.80	80.6	129.1	101.9
HV 9137	2807	52.1	68.3	41.6	57.5	20.00	78.5	125.7	99.2
HV 9139	2744	59.3	73.0	41.7	57.0	22.50	76.7	122.9	97.0
HV 9134	2700	50.3	74.5	41.8	56.3	25.30	75.5	120.9	95.4
CH1[a]	2678	81.6	69.8	37.4	70.2	19.10	74.8	119.9	94.6
HV 9130	2628	57.2	75.5	41.6	59.0	19.80	73.4	117.7	92.9
HV 9110	2606	111.3	74.5	40.6	65.6	15.60	72.8	116.7	92.1
HV 9144	2467	59.9	-	43.5	55.5	18.00	68.9	110.5	87.2
HV 9145	2367	71.0	-	42.7	60.5	15.00	66.2	106.0	83.6
CH2[a]	2233	57.4	68.5	39.9	68.2	18.30	62.4	100.0	78.9
HV 9112	2061	49.0	76.3	39.5	64.3	20.60	57.6	92.3	72.8
HV 9138	2006	55.8	73.5	42.2	62.5	16.50	56.1	89.8	70.9
HV 9142	1836	55.2	59.4	40.9	65.6	18.50	51.3	82.2	64.9
HV 9116	1772	78.4	78.8	41.9	58.8	10.00	49.5	79.4	62.6
Mean	2967	71.0	74.2	41.0	62.9	19.84			
P-Value	<0.001	<0.001	0.045	<0.001	<0.001	<0.001			
CV	14.3	9.5	2.4	1.8	1.6	4.13			
SED	259.7	7.6	1.1	0.2	0.5	0.70			

- =missing data; [a]=check variety

variety, (ii) the lowest performing check variety, and (iii) the mean of check varieties according to Makanda *et al.* (2010) as follows:

Yield advantage over check variety (%) $=[(X_E)/X_{SC}] \times 100\%$

where: X_E =observed mean value of the entry; X_{SC} =mean of the highest check variety or mean of the lowest check variety or mean of the three check varieties.

RESULTS AND DISCUSSION

Agronomic performance of top-cross hybrids

The results indicated a high potential for grain yield among the top-cross experimental hybrids with five hybrids being ranked above the best performing check, CH3, for grain yield across the three countries (Table 1). The yields recorded in this trial (Table 3) are consistent with those reported in the literature (Arshad *et al.*, 2007; de Carvalho *et al.*, 2008; Ghaffari and Farrokhi, 2008; Sepehr *et al.*, 2008) although the highest yielding was just below the highest reported yield of 4.5 t ha^{-1}. Of the five top performing hybrids for grain yield, four had significantly ($P<0.05$) lower 1000 seed weight than the control variety CH3. This suggested that some of the hybrids compensated for lower 1000 seed weight by producing many seeds per head thereby achieving higher yields per plant. This was the case where plant count was not significantly different, for example between the control variety CH3 and hybrid HV9111. Two experimental hybrids HV9120 and HV9132 significantly outyielded the best check variety by 19.6% and 14.5%, respectively (Table 1). Those two hybrids yielded 91.6% and 83.5% more than the lowest check CH2 and 51.2% and 38.1% more than the mean of checks, respectively (Table 1). These values are comparable to those reported in the literature of up to 34% (Gill and Punia, 1996), 66% (Virk, 1984), 89% (Gangappa *et al.*, 1997), but much lower than the highest reported by Manivannan *et al.* (2005) (193%), Singh and Singh (2003) (193%). Further, 16 of the 28 experimental hybrids were not significantly different from the highest performing check variety for grain yield, that is, from HV9136 ranking 17th to HV9120 ranking first in Table 1, further showing high potential for grain yield among the current germplasm.

Oil content was generally high ranging from 36.6% of HV9109 to 44.6% of HV9125. These values are also consistent with those reported in the literature (Keshta *et al.*, 2008; Ghaffari *et al.*, 2011). Eight hybrids, HV9132, HV9141, HV9127, HV9128, HV9125, HV9144, HV9145 and HV9142 had significantly ($P<0.05$) higher oil content than that of the 41.8% of the highest performing check variety CH3 (Table 1). Ghaffari *et al.* (2011) reported similar results, although their figures were on the upper side, up to 48% oil content. The difference can be attributed to the fact that their work used a different set of germplasm and environments as reported by Hladni *et al.* (2008) and Rondanini *et al.* (2003). However, five of these hybrids were not significantly different from the highest oil content check variety CH3 (Table 1). On maturity indicators, days to 50% flowering ranged from 55 to 68 among hybrids and up to 70 for the grain check varieties CH1 and CH2. Of the high yielding experimental hybrids, HV9132, HV9127, HV9128 and HV9135 took less than 60 days to achieve 50% flowering, significantly ($P<0.05$) shorter than the successful cultivars CH1 and CH2 in South Africa. This showed that it was possible to breed early cultivars that are high yielding for Southern Africa from the current germplasm set. Therefore there is potential among the germplasm to breed early cultivars that can outperform the currently successful varieties on the market on grain yield, oil content, and earliness, the major traits of sunflower.

Table 2: Combining ability effects and means of the female parents used in testcross combination evaluated in South Africa, Zambia and Zimbabwe during the 2009/10 rainy season

Female parent	Grain yield				1000 seed weight (g)		Oil (%)		Days to 50% flowering	
	kg ha^{-1}	GCA	g plant^{-1}	g	Mean	GCA	Mean	GCA	Days	GCA
KP1274	2883	-163	63.45	-7.15	80.50	5.83	40.78	-0.60	57.0	-4.76**
KP1316	2006	-1040	55.84	-14.76	73.50	-1.17	42.22	0.84	62.5	0.74
KP1290	3306	260	65.14	-5.46	81.00	6.33	44.62	3.24**	60.8	-1.01
KP1270	3661	615*	66.96	-3.64	71.56	-3.11	40.25	-1.13	66.4	4.64
KP1294	3528	482	74.35	3.75	79.50	4.83	42.58	1.20	58.3	-3.51**
KP1288	3167	121	72.97	2.37	76.00	1.33	40.27	-1.11	68.0	6.24
KP1280	4278	1232**	88.33	17.73**	83.50	8.83	40.50	-0.88	67.0	5.24
KP1331	2467	-579	59.94	-10.66	-	-	43.50	2.12**	55.5	-6.26**
KP1282	3506	460	66.83	-3.77	70.26	-4.41	40.28	-1.10	67.9	6.14
KP1272	1772	-1274	78.39	7.79*	78.75	4.08	41.85	0.47	58.8	-3.01
KP1333	2367	-679	70.98	0.38	-	-	42.70	1.32	60.5	-1.26
KP1296	3483	437	79.43	8.83*	86.75	12.08**	42.50	1.12	57.8	-4.01*
KP1298	3506	460	80.07	9.47*	74.25	-0.42	40.26	-1.12	65.1	3.34
KP1308	2700	-346	50.30	-20.30	74.50	-0.17	41.75	0.37	56.3	-5.51**
KP1300	2628	-418	57.19	-13.41	75.50	0.83	41.58	0.20	59.0	-2.76*
KP1302	3411	365	72.25	1.65	74.80	0.13	40.13	-1.25	65.1	3.34
KP1304	3978	932*	70.68	0.08	75.75	1.08	43.10	1.72*	58.5	-3.26*
KP1312	3165	119	124.20	53.60**	73.72	-0.95	40.36	-1.02	63.7	1.94
KP1310	3172	126	82.97	12.37**	73.75	-0.92	43.10	1.72*	58.3	-3.51*
KP1314	2807	-239	52.08	-18.52	68.33	-6.34	41.60	0.22	57.5	-4.26*
KP1317	2744	-302	59.26	-11.34	73.00	-1.67	41.65	0.27	57.0	-4.76*
KP1320	3419	373	79.50	8.90*	66.62	-8.05	39.83	-1.55	62.9	1.14
KP1235	3706	660*	76.93	6.33	-	-	44.00	2.62**	60.3	-1.51
KP898	1836	-1210	55.23	-15.37	59.40	-15.27**	40.86	-0.52	65.6	3.84
KP471	3111	65	91.41	20.81**	74.99	0.32	40.97	-0.41	66.2	4.44
KP 938	2061	-985	48.97	-21.63	76.25	1.58	39.53	-1.85	64.3	2.54
KP 962	3578	532	62.50	-8.10	69.93	-4.74	36.55	-4.83	67.6	5.86
Mean	3046	0.00	70.60	0.00	74.67	0.00	41.38	0.00	61.8	0.00
SE (GCAs)		285		2.57		2.64		0.50		0.97
SED (GCAs)		403		3.63		3.74		0.70		1.37

*, ** = Parents with desirable and significant GCA effects at 0.05 and 0.01 levels, respectively

\- = missing data

Combining ability effects

The combining ability analysis showed that female parents GCA effects were significant ($P<0.05$) for all the traits. Of the 27 *cms* lines that produced hybrids with sufficient seeds, 16 displayed positive GCA effects for grain yield ha^{-1}, 14 for grain yield $plant^{-1}$, 14 for oil content, 12 for 1000 seed weight while 14 displayed negative GCA effects for days to 50% flowering (Table 2). These results are consistent with the reports of the importance of the additive gene action in controlling the traits (Ghaffari *et al.*, 2011; Khan *et al.*, 2009; Khan *et al.*, 2008; Ashoka *et al.*, 2000; Sassikumar *et al.*, 1999; Alza and Fernandez-Martinez, 1997). Female parents KP1235, KP1304 and KP1290 showed outstanding combining ability performance displaying desirable GCA effects that were significant ($P<0.05$) for at least three of the four major traits, grain yield, 1000 seed weight, oil content and days to 50% flowering (Table 2). These were followed by line KP1280 which displayed high and significant GCA effects for grain yield (both per hectare and per plant) and 1000 seed weight. Its GCA effect for oil content, although negative, was not significant. The line added 5 more days to 50% flowering (significant at 0.05) among its hybrids, making it desirable for high yield but not earliness (Table 2). In general, parents that contributed to a delay in achieving 50% flowering *inter se* generally yielded more than those than contributed to a reduction in the number of days to 50% flowering *inter se*. This is consistent with the general notion that breeding for earliness in plants results in compromised yield levels. However, exceptions to this observation were present. For example KP1294, KP1296, KP1304 and KP1235 displayed positive GCA effects for grain yield together with negative GCA effects for days to 50% flowering of which both GCA effects for KP1304 were significant. This suggested that KP1304 was suitable for breeding for high yield and earliness, a desirable combination for the small-scale and resource-poor farmers in marginal areas of southern Africa. The importance of additive gene action for the traits implied that selection is effective in improving the traits within the current germplasm set. Therefore, the starting point is the selection of good general combiners as parents for the hybrids targeting the region of southern Africa.

CONCLUSIONS

The study identified hybrid HV9120 which significantly out-yielded the highest performing check entry, CH3, and hybrids HV9132, HV9141, HV9127, HV9128 and HV9125, whose yield were not significantly different from that of the CH3 but had significantly higher oil content than the highest performing check for oil content (CH3), as candidate cultivars. These were selected for further evaluation in the region. Additive gene action was shown to be important for grain yield traits, oil content and maturity traits and therefore selection is important in improving these traits. Female lines KP1235, KP1304, KP1290 and KP1280 were identified as good general combiners for use in the development of high performing sunflower hybrids for the region. These will be included in future crosses with many other male parents for hybrid development and population improvement programmes.

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