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Research article

Performances of three-way cross hybrids over their respective single crosses and related heterosis of maize (*Zea mays* L.) hybrids evaluated in Ethiopia

Zemach Sorsa [a,*], Wassu Mohammed [b], Dagne Wegary [c], Amsal Tarkegne [d]

[a] Department of Plant Science, Wolaita Sodo University, Wolaita Sodo, Ethiopia
[b] School of Plant Sciences, Haramaya University, Dire Dawa, Ethiopia
[c] International Maize and Wheat Improvement Centre (CIMMYT), Harare, Zimbabwe
[d] Zambia Seed Company Limited, Ingwezi 11066A, Roma, Lusaka, Zambia

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ABSTRACT

Less attention had been given to the performances of three-way crosses and its comparative advantages of these hybrids over single crosses. This study was carried out to evaluate the performances of three-way crosses in comparison to single crosses for yield and related agronomic traits and to estimate the magnitude of heterosis. The trial was laid out in a simple alpha lattice design of 10 × 6 for lines, 6 × 5 for single crosses (SC), and 9 × 5 for three way-crosses and planted in adjacent plots in the 2019 cropping season in three locations namely Ambo, Abala-Farcha and Melkassa. Single cross hybrids showed a highly significant (P<1%) variation for grain yield, plant height, ear height, and ear length at three locations. These single cross hybrids had showed also a highly significant genotype by environment interaction (P < 1%) for grain yield, plant height, ear height and kernel per ear. Regarding three-way crosses, there was a significant variation (P<5%) on grain yield in Ambo and Melkassa but on ear height and rows per ear in Abala-Faracho. The genotype × environment interaction was significantly varied for grain yield, ear height and ear length. In the comparison, 80% crosses in Ambo, 73% in Abala-Faracho and 67% in Melkassa showed that three-way crosses were better in their performance than that of their respective single crosses. On the other hand, the single crosses that out-performed their respective three-way crosses were higher in Melkassa than Abala-Faracho and the least were reported from Ambo. Similarly, the maximum better and mid-parent heterosis was from single cross 1(769%) in Ambo and single cross 7 (104%) in Melkassa whereas TWC 14 (52%) and TWC 24 (78%) were the highest better and mid-parent heterosis, respectively in Ambo, TWC1 (56%), and TWC30 (25%) were the highest BPH, and MPH, respectively in Melkassa.

1. Introduction

Heterosis is a concept that was proposed by East and defined by Shull before few decades [1,2] and then after many researchers had carried-out research investigating what heterosis is about [3–5], heterotic groups [3,6] and causes of heterosis [6–9] and epigenetic heterosis [10–12]. In the previous study, many countable findings had been added to the science of heterosis, however, the magicness

* Corresponding author.
E-mail address: zemachsorsa@yahoo.com (Z. Sorsa).

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of heterosis is not yet fully addressed, rather continued as the area of research interest for many scientists in different disciplines up to date [9,13]. The research effort on heterosis is progressing up to the speculation of developing inbred lines whose yield performances are expected to be close to elite hybrids without the need for hybrid crossing [6]. When heterosis is considered, the performance and divergence of their inbred parents are of with great importance for developing crosses with high vigoursity [3,14], Single crosses are hybrids that are developed from two genetically different parents of the same species or related species under natural crossing system, similarly, three-way crosses are hybrids which are developed from a single cross (from two different parents) as a female parent with a third parent as a male parent, whereas a double cross is a hybrid which is developed from two single crosses which are made from four different parents in two pair. The possible single, three-way and double-cross hybrids could be developed following the formula below such as $SC = \frac{n(n-1)}{2}$, $TWC = \frac{n(n-1)(n-2)}{2}$, and $DC = \frac{n(n-1)(n-2)(n-3)}{8}$ [15,16]. Whenever heterosis comes to point of discussion, maize (*Zea mays* L.) is one of the cereal crop which is mostly used by different researchers to investigate different types of heterosis like Better Parent Heterosis, and Mid parent Heterosis as suggested by Falconner and his colleague [17]. Single crosses of maize are mostly used to estimate better or mid-parent heterosis however, estimating heterosis from three-way or double crosses is with a full of challenges [18,19].

In the comparison study, single crosses are expressed comparatively a good performance than that of three-way and double crosses [19,20]. In some other study in Canada, single cross out yielded consistently TWC and DC hybrids over two varying locations (Lynch et al., 1973). On contradictory to the above report of significant variation, among three-way crosses and single crosses, grain yield and yield related traits of sorghum showed non-significant variation [21] which indicates it is not matured to generalize the trends of variation among single, three-way and double crosses to all crops. In other study, some of single crosses showed their better performance against the pest resistance [22] indicating the possibility of using single crosses in areas where diseases and pests are serious constraints of maize production. In an experiment which was conducted in the P-defieceint acidic soils, some of single crosses were expressed their efficiency in up taking available phosphorus and showed a high yield performance [23]. For yield and related traits, single crosses were also showed better performance as compared to the Rampur hybrid 2 (single hybrid), check in Nepal [24] and much higher performance as compared to their inbred parents [25].

In the comparison study of single crosses with that of three-way and double crosses, the former has a yield advantage over the others [18,26,27], despite its hybrid seed production cost and its less stability across locations. In the study of single, three-way and double crosses of pepper, single crosses were uniform and performed better for their agronomic traits followed by three-way crosses as compared to double crosses where variation is more prominent accompanied by low performance [19,26], this is not the same for all traits; for some traits TWC out yielded than single crosses [28]. In this regard, three-way cross hybrid has an advantage over single cross hybrids with regards to its good stability and less cost of hybrid seed production [8]. Supporting this hypothesis, in the study of use of sister lines in hybrid seed production, the sister lines which had been developed from a two highly related inbred lines (SLs) were suggested as the best strategy for hybrid seed production [29] when production cost is considered (less acreage required for the production of the same amount of hybrid seed). Moreover, on the comparison study of three-way crosses of tobacco with single crosses, it was reported that the use of three way cross has an economic gain (advantage) [30]. Furthermore, the evaluation of three-way crosses of Arabica coffee report suggested that three-way crosses better performed as compared to single crosses and checks [31], and the mid parent heterosis of lint yield was also reported as the advantage of three-way crosses over single crosses [28].

In addition, environmental variation affects the performances of single, three-way and double crosses of any crop species in different way. Thus, the varying environmental conditions under changing climate, therefore necessitates the application of genotype by environment interaction study for newly developed single, three-way crosses of a given species for their stable performance [32]. Studies showed that the evaluation of single/three-way crosses or double crosses over locations for agronomic traits and its importance of stability become an areas of research interest to easily increase the acceptance and popularity of hybrids [33]. Three-way crosses are with more predictable seed production than seed production with inbred parents [34].

Considering the cost of production of hybrid seed and less stability of single crosses across locations, alternatives are required to bring hybrids with less cost of seed production and wider adaptability across locations. The comparison of three-way crosses with their respective single crosses for quantitative traits and the comparative advantage of three-way crosses were not fully investigated. In this study, the alternative hypothesis that single crosses are not always greater than their respective three-way crosses would be tested by evaluating single crosses with their respective three-way crosses for selected agronomic traits of maize in Ethiopia. Therefore, the objectives of this paper were comparing the agronomic performance and heterosis of single and three-way cross maize hybrids over varying locations for their agronomic traits in Ethiopia.

2. Materials and methods

2.1. The study areas

The research sites consisted of Ambo research center, which is located at 08°57'N latitude and 37°52''E longitude on an altitude of 2225 masl, Melkassa research center is geographically located at of 08°24'N latitude, 39°21'E longitude on an altitude of 1550 masl, and Abala-Farcho, which is lied between 06°55'N latitude and 37°39'E longitude at an elevation of 1,378 masl. Ambo Agricultural Research Center (AARC) is located in West Showa Zone of the Oromia region and this site receives an average rainfall of 1115 mm with the maximum and minimum temperatures of 11.7 and 25.4 °C, respectively and the soil type is clay whereas Melkassa agricultural Research Center is found in the eastern lowlands of Oromia region with andosol soil type which receives the average total annual rainfall of 877 mm, with an average maximum and minimum air temperature of 29 °C and 14 °C, respectively. Finally, Abala-Faracho receives annual rainfall of 50–300 mm with maximum to minimum temperature of 15.5° c to 32°c with the soil type of silty clay.

2.2. Materials

The field evaluation included 58 inbred lines selected based on their yield performance under tropical-mid altitude maize growing condition. In addition, 28 single and 30 three-way cross hybrids were also used in the study. The promising inbred lines were selected purposively and crossed each other to produce heterotic single crosses while the three-way crosses were developed by crossing of the promising single crosses as female parent with the third inbred lines as their male parent. These three trials were tested in adjacent blocks (inbred lines and crosses) in each location by avoiding unequal competition between the hybrids and inbred lines. The evaluation of inbred lines, selecting the inbred lines and developing of single and their respective three-way crosses were carried out by CIMMYT-Harare, Zimbabwe.

2.3. Experimental design and managements

The three-way hybrid trial consisted of 45 genotypes, of which 30 were three-way crosses, 13 were popular commercial cultivars and two were local checks included from the respective locality. This trial was conducted with a 9 × 5 alpha-lattice design (0,1) with three replications. The second single cross trial consisted of 30 genotypes (28 single crosses and 2 local check varieties) which was laid out as 6 × 5 alpha lattice design (0,1) with two replications. The third experimental plot consisted of inbred lines which laid out with a 10 × 6 simple alpha lattice design (0, 1) with two replications. Each entry was planted with a one-row plot of 5.1 m^2 (three-way-crosses, 0.75 × 0.30), 4.25 m^2 (single crosses; 0.75 × 0.25) and 4.00 m^2 (inbred lines; 0.75 × 0.25) lengths between rows and between plants. All the recommended agronomic packages in respective locality were applied to all trials and finally the treatment was managed under rain fall conditions where there was no moisture stress at emergence in all locations.

2.4. Data collection and analysis

2.4.1. Data collection

Plant height (cm) was measured from 10 randomly taken plants by measuring from the base to the tip of tassel or flower of maize plants whereas the ear height (cm) was measured from the base of the plants to the tip of silk. Ear length (cm) was measured by taking ten ears starting from the base of the ear to the tip of the ear. Number of rows per ear (count) was measured by counting the number of row per ear from ten plants and then the average was considered for further analysis. Similarly, number of kernels per row (count) was measured by counting the number of kernels per row taken from ten plants and the average was used for further analysis. Finally, grain yield (tha^{-1}) was measured using sensitive balance (kg) from ten plants in each plot, later the average converted into tha^{-1} and used further analysis.

2.4.2. Data analysis

The agronomic data collected was subjected to analysis (ANOVA) using R package for multi-environment trails [35] where the significance at individual location and over locations were carried out for each set of experiments (parents, single crosses and three-way crosses) (Supplementary Table 1). Normality of the distribution of variance for the traits was tested using R package applying Shapiro-Wilk normality test before combined analysis [36] and the data was normally distributed. Comparison of mean was done by least significant difference test (LSD) at 5% of level of significance. Genotype by environment interaction analysis was conducted by applying GenStat version [37]. The comparison of better-parent, mid-parent, and standard heterosis of grain yield for single and three-way hybrids were done for Ambo and Melkassa, however, only the standard heterosis was computed for Abala-Faracho, which was due to lack of data from inbred lines caused by drought during early growth period. As the procedure, the effect of heterosis for each trait was estimated by the comparison of mean of a particular single cross hybrid with its parents whereas the three-way hybrid was compared with its direct parents (single cross (female parent) and third parent (line)). Better parent (BP) and Mid parent (MP) Heterosis were calculated according to the formula suggested by Falconer [17]:

$$BPH\ (\%) = \frac{F1 - BP}{BP}\ x\ 100,$$

$$MPH\ (\%) = \frac{F1 - MP}{MP} x100, \text{ and}$$

$$SH\ (\%) = \frac{F1 - SC}{SC} x100,$$

Where F1 = Mean value of the cross.

BP = Mean value of better parents.

MP = Mean value of the two parents.

SC = Mean value of Standard checks.

Significance of heterosis was tested using the *t*-test against the critical difference (CD). How to find critical difference (CD) for BPH, MPH, and SH was suggested by Cochran and Cox [38]and more detailed by Ref. [39] as stated below.

Table 1
Overall mean performances, heritability, genotype and genotype x environment variances estimated for eight traits of single crosses evaluated at three locations in 2019/20 cropping season in Ethiopia.

	Statistic	GY	PH	EH	EL	ED	RPE	KPR	TSW
Ambo	Heritability (%)	82	88	91	80	23	55	77	78
	Genotype Variance	1.76**	212.36**	179.81**	1.88**	0.04^{NS}	0.41*	6.75**	1462.4**
	Grand Mean	8.25	224.20	115.23	16.86	4.87	13.68	36.53	339.17
	LSD (5%)	1.84	17.47	13.92	2.06	1.08	1.71	4.14	64.22
	CV (%)	10.64	3.34	5.41	5.17	10.57	5.98	5.41	8.04
Abala-Faracho	Heritability (%)	84	82	66	28	9.4	36	79	50
	Genotype Variance	0.66**	353.61**	1.77*	0.45^{NS}	0.004^{NS}	0.33^{NS}	43.47**	1001.16^{NS}
	Grand Mean	2.49	144.99	21.25	17.12	4.18	13.08	25.12	262.93
	LSD (5%)	1.04	22.95	3.26	1.69	0.56	2.29	10.48	101.81
	CV (%)	19.99	7.80	6.54	8.80	6.44	8.36	19.94	17.49
Melkassa	Heritability (%)	56	74	71	76	43	55	74	0.001
	Genotype Variance	0.08*	216.17**	183.94**	1.68**	0.02^{NS}	0.32*	11.22**	0.001^{NS}
	Grand Mean	1.92	231.67	112.00	17.12	4.18	13.79	35.25	262.93
	LSD (5%)	0.77	30.16	27.32	2.17	0.49	1.51	5.87	135.63
	CV (%)	19.06	5.51	10.67	5.87	5.64	5.22	7.96	24.65
Combined	Heritability (%)	55	89	66	36	1.2	56	42	65
	Genotype Variance	0.29*	235.54**	60.09**	0.31*	0.003^{NS}	0.17^{NS}	5.64*	826.75*
	Gen x Env Variance	0.54**	25.18^{NS}	63.89**	0.84**	0.03^{NS}	0.001^{NS}	17.09**	306.46^{NS}
	Grand Mean	4.18	202.34	82.12	16.94	4.4	13.58	32.38	296.93
	LSD (5%)	1.41	16.25	16.26	2.11	0.50	1.05	7.85	65.54
	CV (%)	14.91	5.59	9.43	7.46	7.77	6.66	10.65	12.28

**, and * highly significant and significant at 5% level of significance, GY = grain yield, PH = Plant height, EH = ear height, EL = Ear length, ED = ear diameter, RPE = row per ear, KPR = kernel per row, and TSW = thousand seed weight.

i) Critical difference for heterosis over better parents (BPH):

CD for BPH $= \pm(\sqrt{(2eMS/r)}$ x t

SE (d) for BP $= \pm\left(\sqrt{\left(\frac{2eMS}{r}\right)}\right.$,

t (better parent) $= \frac{F1-BP}{SE\ (d)}$,

ii) Critical difference over standard Variety.

CD for SH $= \pm(\sqrt{(2eMS/r)}$ x t

SE for SH $= \pm\left(\sqrt{\left(\frac{2eMS}{r}\right)}\right.$,

t (Standard Variety) $= \frac{F1-SC}{SE\ (d)}$,

Where SE (d) is standard error of difference, eMS is the error mean square in the replications; F1, BP, MP and SC are mean values of F1, better parent, mid parent, and Standard variety, respectively. Critical differences were calculated at 5% of level of significance.

2.4.3. Heritability analysis

- Heritability (broad sense), $H^2 = \sigma^2_P / \sigma^2_g$, where σ^2_P = phenotypic variance, and $\sigma^2 g$, = genotypic variance.

3. Results and discussions

3.1. Analysis of variance and heritability for agronomic traits of maize hybrids

The analysis of variance at Abala-Faracho site exhibited highly significant difference (P > 1%) for grain yield (GY), plant height (PH), and kernel per row (KPR), for ear height (EH) and thousand seed weight (TSW), and a significant difference (P > 5%) for row per ear (Table 1). In this environment, hybrid H21 (4.33tha^{-1}) followed by H16 (4.29tha^{-1}) were the highest yielders whereas hybrid H20 (1.04 tha^{-1}) was the lowest yielder (see Appendix Table 1). Moreover, 50% of the hybrids performed above the mean grain yield (2.49 tha^{-1}) in this environment. In Ambo, from traits studied, all growth and yield related traits except ear diameter had showed a highly significant variation and from yield related traits, only row per ear expressed a significant difference among treatments (Table 1). The performance of yield related traits varied from 10.1 to 14.6 for RPE, 7.51–37.99 for KPR, and 188–381 for TSW. Likewise, the performance of growth related traits showed variation of 108–190 for plant height, 41–98 for ear height, and 14–19 for ear length (see Appendix Table 1). Similarly, in Abala-Faracho and Melkassa grain yield and kernel per row from yield related traits, and plant and ear height from growth related traits had commonly showed a highly significant and significant difference (P > 1%/5%) (see Table 1).

Heritability was estimated ≥50% for majority of traits studied except ear diameter at Ambo, ear length, ear diameter and row per ear at Abala-Faracho, and ear diameter and thousand seed weight at Melkassa. The highest heritability for grain yield was obtained from Abala-Faracho (84%) and the lowest was from Melkassa (56%), and the heritability of other studied traits expressed with different magnitudes across locations (Table 2). Heritability estimated over locations was high for plant height 89% and ear height

Table 2
Overall mean performances, heritability, genotype and genotype x environment variances estimated for eight traits of three-way crosses evaluated at three locations in 2019/20 cropping season in Ethiopia.

	Statistic	GY	PH	EH	EL	RPE	KPR	TSW
Ambo	Heritability (%)	73	1.5	36	4	13	1.2	9
	Genotype Variance	2.31**	0.01^{NS}	85.45^{NS}	0.03^{NS}	0.10^{NS}	0.01^{NS}	30.04^{NS}
	Grand Mean	9.96	245.96	130.71	16.52	15.42	38.23	350.66
	LSD (5%)	2.70	40.07	35.30	2.33	2.84	4.65	60.19
	CV (%)	13.23	7.41	12.29	5.76	8.16	4.91	7.03
Abala-Faracho	Heritability (%)	20	1.9	9	34	46	4	1.8
	Genotype Variance	0.11^{NS}	0.001^{NS}	17.79**	1.18^{NS}	0.23*	0.06^{NS}	0.01^{NS}
	Grand Mean	2.80	165.95	74.99	23.38	13.47	33.36	325.09
	LSD (5%)	2.10	38.43	5.24	5.31	1.63	4.59	123.25
	CV (%)	33.56	11.09	2.93	9.39	4.84	5.44	18.17
Melkassa	Heritability (%)	52	6	55	73	0.09	0.01	31
	Genotype Variance	0.07*	8.08^{NS}	84.38**	1.08**	0.001^{NS}	0.002^{NS}	28.78^{NS}
	Grand Mean	1.86	229.28	114.02	16.80	14.49	35.50	220.57
	LSD (5%)	0.72	33.10	23.98	1.89	1.59	4.51	27.47
	CV (%)	17.59	7.05	10.27	5.41	4.47	5.12	5.19
Combined	Heritability (%)	0.08	0.01	13	0.06	50	12	8
	Genotype Variance	0.001^{NS}	325.4^{NS}	7.45^{NS}	0.82^{NS}	0.22*	13.51^{NS}	14.22^{NS}
	Gen x Env Variance	0.79**	287.6^{NS}	55.05**	12.79*	0.008^{NS}	0.68^{NS}	14.32^{NS}
	Grand Mean	4.83	213.8	110.02	18.93	14.45	35.71	286.30
	LSD (5%)	1.84	20.97	19.35	2.48	1.49	4.9	32.10
	CV (%)	19.42	8.6	11.56	7.93	6.45	12.0	6.42

**, and * highly significant and significant at 5% level of significance, GY = grain yield, PH = Plant height, EH = ear height, EL = Ear length, ED = ear diameter, RPE = row per ear, KPR = kernel per row, and TSW = thousand seed weight.

66%, moderate for kernels per row 56%, thousand seed weight 55% and grain yield 55%, but it was low less than 50% for ear length, ear diameter, and kernels per row. This variation in the expression of heritabilities' of different traits might be related to the degree of conducive growth period and the abilities of each hybrid to withstand constraints in the growth environment. Broad sense heritability varies among agronomic traits and also between varying growing conditions where it was high to moderate in favourable growing condition and the opposite in the low lands [19,20,40].

The combined analysis of variance over locations showed significant differences among single crosses for yield and other related traits except for ear diameter and row per ear. Genotype × environment interaction (GEI) was highly significant for grain yield, ear height, ear length and kernels per row (Table 1). This findings was in line to other previous report [19,32,41] where single and three-way crosses showed a significant variation for agronomic traits. Over all mean grain yield performance of single cross and three-way cross was demonstrated on the figure (Fig. 1). From the Fig. 1, it indicated that mean grain yield of three-way crosses at Ambo was higher than the mean grain yield of single crosses. On the other hand, the mean grain yield performance of three-way crosses in Abala-Faracho and Melkassa showed a comparable performance to their respective mean grain yield of single crosses in each location (see Fig. 2).

With regards to three-way crosses, the ANOVA for only grain yield was highly significant ($P < 1\%$) in Ambo, and significantly ($P < 1\%$) different in Melkasssa, whereas the genotypic variation was not significant in Abala-Faracho and in the combined analysis. However, the combined analysis was significant ($P < 5\%$) for number of rows per ear across locations. Likewise, the analysis of genotype by environment interaction was highly significant ($P<1\%$) for grain yield in the combined analysis, followed by a significant interaction ($P<5\%$) for ear height, and ear length in Melkassa as well as in the combined analysis. In Abala-Faracho, the analysis of variation of three-way crosses were not significant for majority of traits except for ear height ($P<1\%$), and number of row per ear ($P<5\%$). Comparatively, more number of agronomic traits showed a significant variation in single crosses than three-way crosses (Geleta & Labuschagne, 2004; Lynch et al., 1973). Heritability with regards to three-way crosses was only good in Ambo ($H^2b = 73\%$) and Melkassa ($H^2b = 52\%$) for grain yield. Moreover, it was also good for ear height and ear length in Melkassa ($H^2b > 50\%$). This indicates heritability declines from high favourable to less favourable growing environments for some of agronomic traits and shows a decrease of it from single crosses to three-way crosses [18,30]. If we consider only grain yield for discussion, there was a declining trend of variation for agronomic traits from high land to lowland and similar trend of variation was obtained from single crosses to three-way crosses, this might be due to the genetic mechanism of heterosis potential in single crosses than three-way crosses [9,20].

The genotype × environment interaction was highly significant for more number of agronomic traits in the single crosses than three-way crosses indicated that the three-way crosses were more stable than single crosses similar to the previous report on sorghum [32]. This also might be due to the fact that the difference in the genetic mechanism of three-way crosses than single crosses [9,30,42]. However, as the genetic background broadness increases, the logic expects superer performance or maximum heterosis for some agronomic traits but the reality resulted in stability than increment. This might be due to adaptive gene expression exchange that lead to the performance variation between single crosses and their counterpart three-way crosses [43].

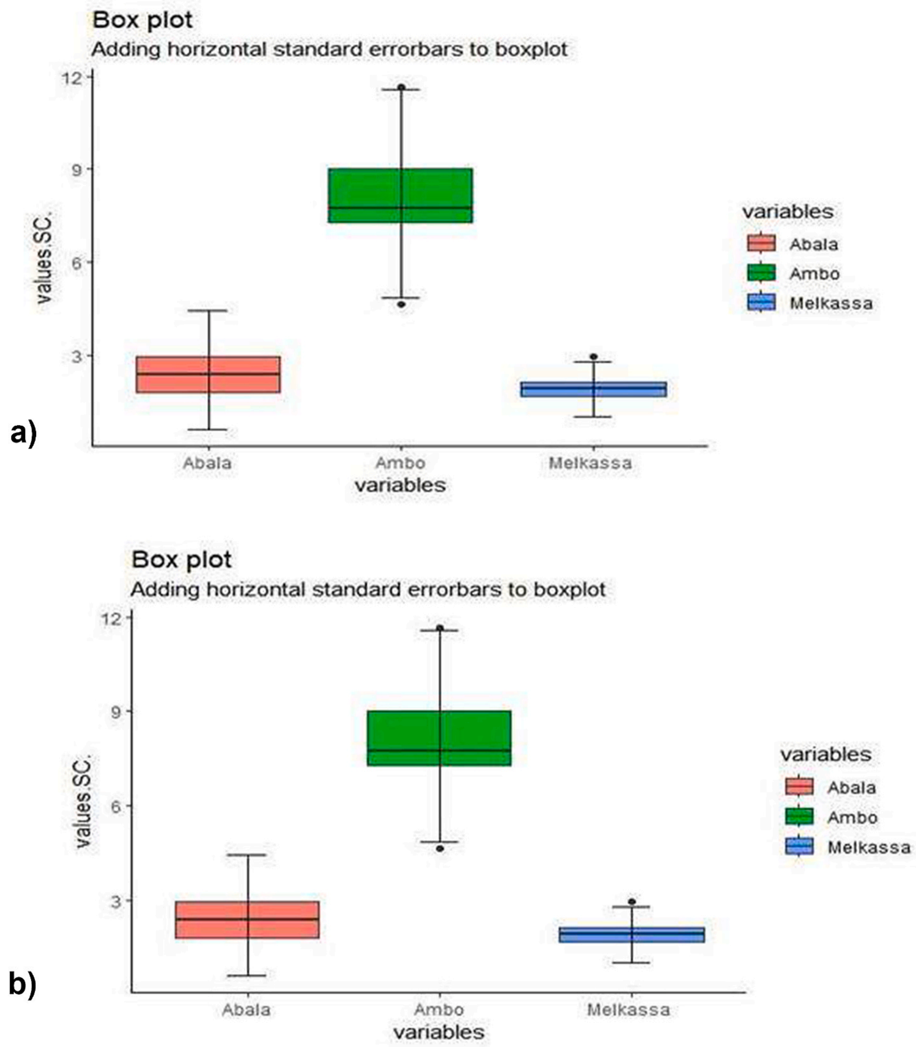

Fig. 1. Overall mean grain yield performances of a) single and b) three-way crosses over three locations in Ethiopia evaluated in 2019 cropping season.

3.2. Overall mean performances of single and three-way crosses for grain yield

The overall mean grain yield performances of single and three-way crosses were compared in the following manner where performance at Ambo was higher for both types of crosses, and the trends of variation was not similar, rather comparable for both types of crosses in Abala-Farcho and Melkassa (Fig. 1).

3.3. Range and mean performances of hybrids for agronomic traits

The single crosses had mean grain yield of 8.15, 2.67 and 1.98 t ha^{-1} at Ambo, Abala-Farcha and Melkassa, respectively, that ranged from 1.04tha^{-1} (Melkassa) to 11.9 t ha^{-1}(Ambo). The three way-crosses had lowest and highest mean grain yield of 1.98 and 10.04 t ha^{-1} at Melkassa and Ambo, respectively, with mean grain yield of 10.04, 2.75 and 1.98 tha^{-1} at Ambo, Abala-Farcha and Melkassa, respectively (Table 3). Theese three-way crosses had mean grain yield advantages of 189, 8 and 6% over single crosses at Ambo, Abala-Farcha and Melkassa, respectively. Similarly, the yield advantage of three-way crosses over single crosses was 153% for plant height, 1675% for ear height, 162% for rows per ear, 144% for kernel per row. However, in contrast to the above the yield advantage of single crosses over three-way crosses was 46% for ear length and 551% for thousand seed weight at Ambo. Likewise, all the traits expressed a yield advantage of three-way crosses over their single crosses in Abala-Faracho and Melkassa except for thousand seed weight in Melkassa where it showed a yield advantage of single crosses (4956%) over three-way crosses(seeTable 4). The broad genetic background in three-way crosses might help to withstand the harsh growing conditions in the lowland than to their counterpart (single crosses) in the same growing condition that might be due the role of genetic mechanisms' differences in both crosses [43,44],

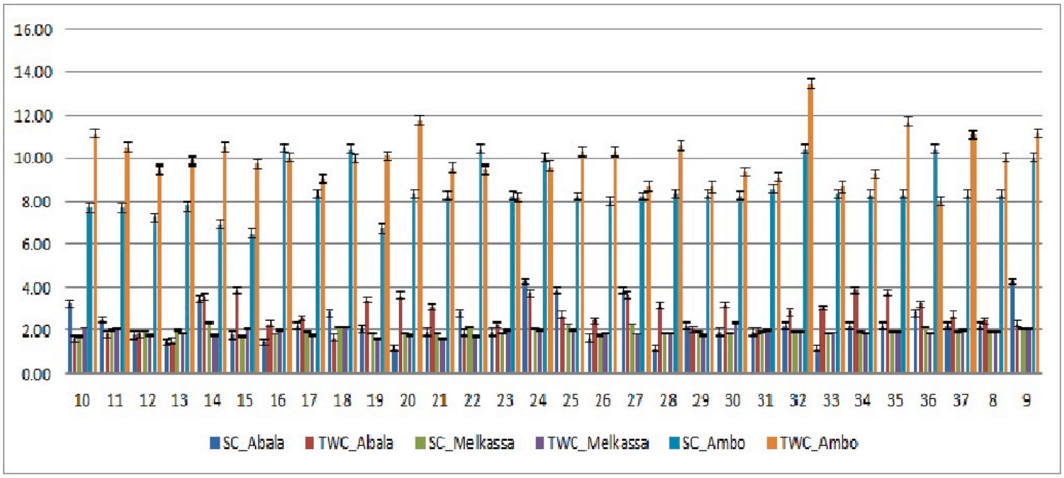

Fig. 2. Comparison of grain yield (t/ha) of three-way crosses with their respective single crosses evaluated at three locations. SC= Single Cross, TWCs = Three-Way Crosses.

Table 3
Range and mean performances of only single and three-way crosses for seven agronomic traits evaluated at three locations in Ethiopia during 2019 cropping season.

Genotype	Location	Variable	GY	PH	EH	EL	RPE	KPR	TSW
Single crosses	Ambo	Range	4.6–11.9	180–256	71–143	11–20.3	9.3–15.3	26–46.33	250.9–448.9
		Mean	8.15	222.8	114.12	16.71	13.76	36.45	333.68
		SD	0.78	56.7	32.38	0.79	0.68	3.9	759.86
		CV (%)	10.81	3.38	4.99	5.33	6.00	5.42	8.26
Three-way crosses		Range	5.5–15.1	200–280	80–180	11.5–19.2	10.12–19	28–47.8	250.9–389.5
		Mean	10.04	243.11	130.85	16.25	15.38	37.89	328.17
		SD	1.37	204.56	171.10	0.81	1.90	3.05	638.12
		CV (%)	11.66	5.88	10.00	5.53	8.95	4.61	7.70
Single crosses	Abala-Faracho	Range	1.2–4.4	94.7–199.1	41.4–89.7	11.8–19.8	7.4–15	7.3–38.4	143.4–408.2
		Mean	2.67	153.47	73.89	17.08	4.17	13.11	262.93
		SD	0.02	155.73	5.48	2.34	0.08	1.25	24.75
		CV (%)	4.76	8.13	3.17	8.96	6.61	8.53	19.64
Three-way crosses		Range	0.52–5.23	126.2–193	65.9–103.8	10.2–36.3	11–17.5	23.7–39.5	234.5–398
		Mean	2.75	167.22	84.52	23.65	13.54	33.46	327.78
		SD	0.69	147.21	43.87	2.66	0.28	4.73	46.87
		CV (%)	30.35	7.26	7.84	6.90	3.88	6.50	2.09
Single crosses	Melkassa	Range	1.04–3.04	172–282	66–148	11.8–20.2	11.6–15.6	25.2–45.2	179.1–358.2
		Mean	1.98	230.56	111.11	17.02	4.19	13.89	269
		SD	0.04	127.11	129.14	0.97	0.06	0.56	7.94
		CV (%)	10.19	4.89	10.23	5.79	5.62	5.37	7.96
Three-way crosses		Range	1.25–3.07	200–266	86–142	14.5–19.8	10.8–16.8	25.4–45.2	189.5–258.9
		Mean	2.04	231.35	113.33	17.06	14.54	35.48	219.44
		SD	0.13	332.26	130.68	0.67	0.50	0.68	84.16
		CV (%)	17.94	7.88	10.09	4.79	4.88	2.32	4.18

GY (t ha^{-1}) = grain yield, PH (cm) = Plant height, EH (cm) = ear height, EL (cm) = Ear length, RPE = number of rows per ear, KPR = number of kernels per row, and TSW (g) = thousand seed weight. SD = standard deviation and CV (%) = percentage of coefficient of variation.

and this was similar to the previous study on upland cotton where the proportion of dominance and additive genetic variance for yield component was higher in three-way and double crosses than in single crosses [19,28] .

Moreover, the mean of checks of single crosses was 4.99 tha^{-1} and that of three way-crosses was 4.72 tha^{-1} over locations (Appendix Table S1). A total of five single crosses such as SC16, SC21, SC10, SC2, and SC22 had higher mean grain yield than the best check SC30 (4.99 tha^{-1}) across locations while 3 three-way-crosses such as TWC20, TWC 32, and TWC 35 had higher mean grain yield than the best check TWC 41(5.9 tha^{-1}). In addition, twelve hybrids had shown higher yield performance than that of the mean grain yield of single crosses (4.18 tha^{-1}) across locations while fifteen hybrids were yielded above the mean grain yield of three way-crosses across locations (4.83 tha^{-1}). There were many previous study comparing single crosses and three-way crosses with their respective check similar to this finding found that some of the hybrids performed better than their checks [19,21,23,28,29].

Table 4
Estimate of heterosis for single and three-way crosses for grain yield at three locations in Ethiopia in 2019.

Location	Type of crosses	Heterosis (%)	Range (%)	Mean of heterois	CD Calculated	CD (5%)	CD (1%)
Ambo	Single	Mid parent	12.68–769.84	287.32	0.84^{NS}	1.80	2.43
		Better parent	−5.86–743.18	212.42	5.18**	1.80	2.43
		Standard	−51.28–13.26	−16.27	-1.59^{NS}	1.80	2.43
	Three-way	Mid parent	23.48–77.91	53.00	1.96**	2.61	3.48
		Better parent	−23.00–52.00	19.00	1.51^{NS}	2.61	3.48
		Standard	−24.43–40.40	2.71	0.30^{NS}	2.61	3.48
Melkassa	Single	Mid parent	−19.43–168.52	53.61	0.01^{NS}	0.41	0.56
		Better parent	−25.59–148.85	40.22	0.52**	0.41	0.56
		Standard	−24.21–48.24	0.45	-0.01^{NS}	0.41	0.56
	Three-way	Mid parent	−9.59–56.50	16.00	0.45^{NS}	0.74	0.98
		Better parent	−25.00–25.00	−1.00	0.07^{NS}	0.74	0.98
		Standard	- 8.30–67.70	−33.89	0.51^{NS}	0.74	0.98

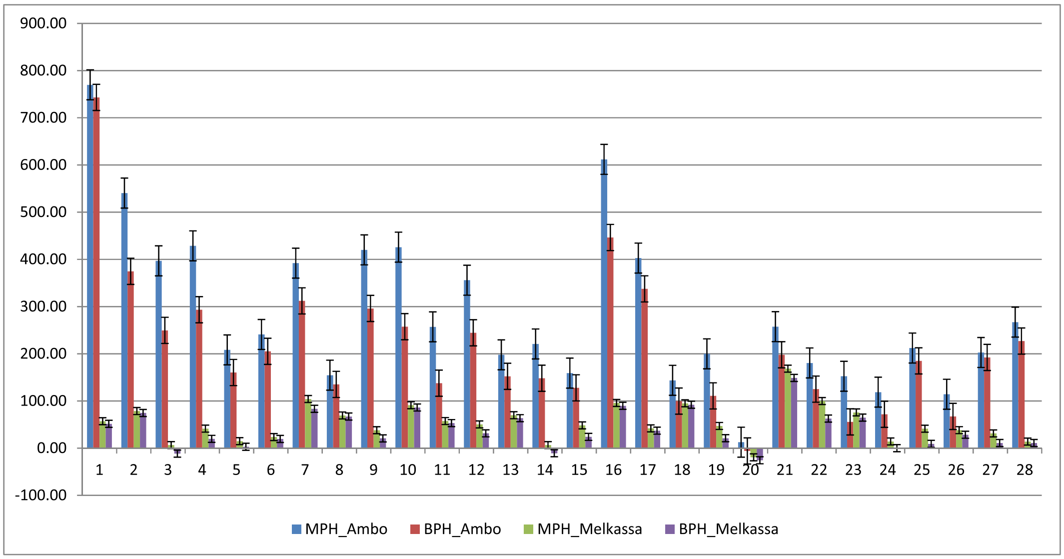

Fig. 3. Comparison of Mid and Better parent Heterosis of single cross hybrids at Ambo and Melkassa. MPH = Mid Parent Heterosis, BPH= Better Harent Heterosis.

3.4. Comparison of grain yield of single crosses with their respective three-way crosses for grain yield performance across locations

In the figure below, majority of the three-way crosses especially in the Ambo performed above their respective single crosses for grain yield, however few single crosses involved in the development of the following three-way crosses: TWC16, TWC18, TWC22, TWC23, TWC24, and TWC36 performed above their respective three-way crosses. The highest yielder in this location was TWC32 from three-way cross while SC10 from single crosses. The single crosses which performed above their three-way crosses were SC28 in TWC16, SC10 in TWC18, SC10 in TWC22, SC4 in TWC23, SC16 in TWC 24, and SC10 in TWC36, respectively. In similar way, majorities of three-way crosses out performed their respective single crosses at Abala-Faracho except for SC15 in TWC10, SC24 in TWC11, SC10 in TWC18, SC10 in TWC22, SC16 in TWC24, SC22 in TWC25, SC22 in TWC27, SC16 in TWC9. Likewise, majority of three-way crosses in Melkassa outperformed their respective single crosses except for few single crosses (better than in other locations) such in SC19 in TWC12, SC13 in TWC13, SC18 in TWC14, SC9 in TWC17, SC6 in TWC19, SC4 in TWC21, SC10 in TWC22, SC22 in TWC25, SC 22 in TWC27, and SC10 in TWC36. In general the single crosses that out-performed their respective three-way crosses were higher in Melkassa than in Abala-Faracho and the least were reported from Ambo. However, the majority of three-way crosses over single crosses in all locations indicated the presence of comparative advantage of three-way crosses over single crosses for grain yield trait. In the previous study, less attention was given to this comparative advantage of three-way crosses over their respective single crosses. This study supports to lesser extent the previously known finding where the single crosses had a yield advantage over their respective three-way or double crosses, however, to more extent highlights the presence of grain yield advantage of three-way crosses over single crosses [28]. Moreover, this was in line with the previous report where the higher proportion of dominance to additive genetic variance for yield component of upland cotton that exists in three-way and double crosses than in single crosses [19,28]. The three-way and/or other multiple cross hybrids were expected to provide the consumers with higher quality traits like minerals in addition to comparable quantitative traits than single crosses [19,29,45].

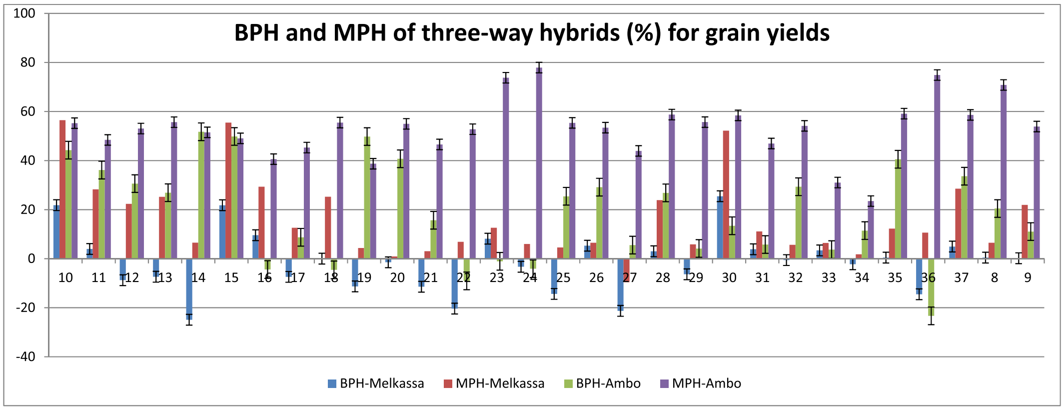

Fig. 4. Mid-parent and Better-parent heterosis of three-way crosses at Ambo and Melkassa. MPH = Mid Parent Heterosis, BPH= Better Harent Heterosis.

In this study about 80% in Ambo, 73% in Abala-Faracho and 67% in Melkassa of hybrid combinations showed that TWCs were better in their performance than that their respective SC, this is in contrast to the previous report [20] where no obvious difference was observed among the categories of single, three-way and double crosses of tropical maize hybrids. On the other hand, similar to this finding, high variation was reported [19] where three categories of hybrids of pepper performed differently and showed high variation in three-way crosses for the majority of traits compared to single crosses. Moreover, three-way crosses showed a better thermal tolerance than single crosses[46] . On the other hand, on sorghum crop it was reported that the three-way hybrids were significantly greater than those of single-crosses, but the differences suggested as not large enough to say agronomically important [21]. Three-way crosses combinations were suggested not only for quantitative expression of agronomic traits like for grain yield but also for qualitative traits like for minerals [28,45], indicating the possibility of increasing mineral yields through TWCs and the likes without losing its advantage of agronomic yields. The three-way top cross hybrids of per millet were found to be better than other types of hybrids in performing for quality traits maintaining comparable performance of quantitative traits [47]. From the analysis, there is an obvious yield advantage from majority of TWCs over single cross hybrids for grain yield which is definitely in contrast to the previous report [26] where single crosses out yielded the other crosses in both high and low yielding environments. In addition, better anther culthurability of indicia rice was also reported from three-way crosses than single crosses [48].

4. Heterosis

Most of the single cross hybrids revealed a positive and highly significant better-parent ($P \leq 1\%$) heterosis whereas a positive and highly significant mid-parent heterosis for three-way crosses at Ambo. The maximum better and mid-parent heterosis was observed from the single cross 1 (769%), followed by H16 (612%) at Ambo (Fig. 3). At Ambo eighty two (82) percent (23 out of 28) of single cross hybrids showed their both types heterosis (BPH and MPH) above hundred percent. Similarly, majority of single cross hybrids expressed a positive and highly significant better-parent heterosis ($P \leq 1\%$) for grain yields at Melkassa. However, the analysis was not significant for all types of heterosis (Mid-parent, Better-parent and standard heterosis) regarding three-way crosses at Melkassa. In this location, the maximum mid and better parent heterosis was obtained from SC7, and SC21, these all showed its performance inline to the previous study report [18,49]. However, in this location twelve single crosses (43%) had expressed both types of heterosis above fifty percent (>50%). In addition, few hybrids SC20 from Ambo, and SC3, SC5, SC14, and SC20 from Melkassa had expressed a negative heterosis (both type) for grain yield. Within the same environment whether in Ambo or Melkassa the genotypes (hybrids) showed a different heterosis which might be due to the genetic difference that caused their performance [7] and also due to heterotic gene that might elevate crop yields [10].

When their critical difference was considered, better parent heterosis of single crosses and mid-parent heterosis of three-way crosses in Ambo had showed a highly significant variation ($P<1\%$) where as in Melkassa only better parent heterosis of single crosses had revealed a highly significant variation. This observed differences might be due to the genetic and environmental influences that affected the agronomic performance of both types of hybrids [8,20,40,50].

4.1. Better and mid - parent heterosis of three-way cross hybrids

In the comparison of better and mid-parent heterosis, majority of both types of heterosis were positive at Ambo while TWC 24 (78%) and TWC14 (52%) were the highest mid-parent and better parent heterosis, respectively. The following top crosses TWC10, TWC11, TWC12, TWC 14, TWC 15, TWC19, TWC20, TWC 26, TWC35, and TWC37 were the best hybrid with both types of heterosis above 30%. On the other hand, more than ten hybrids at Melkassa had expressed a positive heterosis of both types while TWC1 (56%), and TWC30 (25%) were the highest BPH, and MPH, respectively in this site. From top ten both types of heterosis, majority of them were between 4 and 20% of heterosis except for TWC10, and TWC30 (≥20%) (Fig. 4). From the study on wheat supporting in favor of

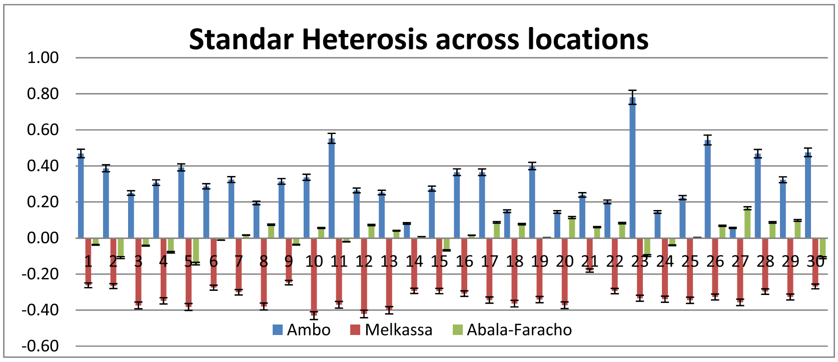

Fig. 5. Comparison of standard heterosis of three-way hybrids for grain yield in Ethiopia.

this paper, there was a report indicating the existence of high-parent heterosis where TWCs had showed high parent heterosis [51]. The higher the difference between BPH and mid-parent heterosis, then the best advantageous would be the use of BPH involving the single crosses in the development of three-way crosses (TWCs). This is due to the fact that the better parent performance was higher than mid parent performance of inbred parents, hence the lines combination of single crosses which results in BPH when considered as a female parent would have a potential to increase the productivity (heterosis) of resulting three-way crosses. This might be due to the reason that parents with high per se performance and intermediate genetic divergence expected to produce highly heterotic and high yielding hybrid (Heterosis) [52]. On the other hand, better or mid-parent heterosis of TWCs for grain yield at Melkassa showed low performance differing from Ambo (Fig. 4). Thirty-nine percent (eleven hybrids) of the TWCs in this location were with positive better parent heterosis (TWCs > SCs) and the remaining showed negative better parent heterosis indicating that single crosses (better parents) were higher in performance than three-way crosses (TWCs < SCs). This higher better parent heterosis of TWCs than SC for grain yield in this study is in line to the previous report for lint yield of cotton [28] where TWCs performed better than single crosses for yield traits in upland cotton cultivars.

4.2. Standard heterosis

The highest standard heterosis was obtained from the hybrids' performance in Ambo followed by Abala-Faracho and the least and negative standard heterosis was obtained from hybrids' performance in Melkassa (Fig. 5). So, the maximum standard heterosis at Ambo was about 78% (TWC23), 17% (TWC 27) at Abala-Faracho, and −43% (TWC10) at Melkassa. The result indicated that there was high interaction for standard heterosis across locations; this might be primarily duet to the genetic variation and secondly due to the existing environmental variation [53,54]. Similar to this finding, in other study report it was indicated that the degree of heterosis varies not only based genetic expression of specific trait, but also on the environment where that trait is measured [55]. In such a diverse environment the potential use of selected heterozygous hybrids was suggested to mitigate losses arising out of climate change [56]. Hence proposing the variety release verification test or direct use of the high performing hybrids at Ambo or Abala-Faracho/Melkassa would be economically advantageous for the end users (farmers). With this regard, TWC23, TWC11, TWC26, TWC1, TWC28, and TWC30 were the three-way cross hybrids which expressed the standard heterosis more than 40% in Ambo. Likewise, TWC27, TWC20, TWC29 and TWC28 were relatively the best hybrids for end users in Abala-Faracho. Previous reports on standard heterosis [53,54,57] supported the finding of this paper indicating the potential of TWCs for direct use or for further breeding program in these contrasting environments.

5. Conclusion

Unlike the previous findings of various study comparing single crosses with three-way crosses, in this study majority of three-way crosses had showed a significant yield advantage over their respective single crosses of maize for grain yield. Except the extent of variation in magnitude, the three-way crosses outperformed their respective single crosses in both locations. In the comparison of mid and better parent heterosis of single crosses for grain yield the expression of heterosis at Ambo was better than that of Melkassa. However, the expression was not consistent for majority of single crosses except for SC18 and SC21 where the expression for both types of heterosis to some extent was consistent over locations (Ambo vs Melkassa).

With regards to heterosis of three-way crosses, majority of TWCs' heterosis was positive and thus TWCs whose better parent heterosis was positive would be with a great potential for further research on the single crosses which were a better female parent in the development of three-way crosses. It implies that high number of seeds from single cross (better female parent of TWCs) would lead to get predicted amount of TWCs by minimizing the cost of seed production that would be very important for seed companies in developing countries. Development of male sterile single cross (female parent of TWCs) would be the research direction for efficient utilization of TWCs in the future.

Finally, further testing of the selected hybrids in the target environments (Ambo, Abala-Faracho and Melkassa) would be advisable in solving the stability of grain yield.

Author contribution statement

Zemach Sorsa: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Wassu Mohammed: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Dagne Wegary; Amsal Tarekegne: Conceived and designed the experiments; Contributed reagents, materials, analysis tools, or data.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e15513.

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