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Research Article

Genetic improvement of palmarosa (*Cymbopogon martini* var. motia) for herbage yield and essential oil quality through polycross system of breeding

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Abstract

Palmarosa is one of the commercially important essential oil yielding crops of India. Improving the herbage and essential oil yield through appropriate breeding methods is the major objective of this crop. Being a cross pollinated crop, its improvement can be made through mass selection. Fifteen best palmarosa clones are identified from two years of individual plant screening of seven germplasm material for different growth, yield and quality parameters formed the base material for this study. The slips of fifteen selected clones were planted in the polycross nursery at ICAR-DMAPR, Anand, Gujarat. The composite hybrid seeds of palmarosa were collected separately from each clone from the polycross nursery. In the present study, the performance of composite hybrids along with check was evaluated to identify best performing polycross hybrids for herbage and essential oil yield. Two hybrids DCMH 8 and DCMH 10 had more than 22 per cent higher dry herbage yield than the commercial variety Trishna. The polycross hybrid progeny proved to be significantly superior with respect to essential oil content and percentage of essential oil recovery. Among the hybrids DCMH 8 had the highest oil yield (16.08 g/plant), which was 37.79 per cent higher than check variety. Based on the performance of the polycross composite hybrids, top performing five hybrids viz., DCMH 1, DCMH 6, DCMH 8, DCMH 10, and DCMH 14 were selected and advanced to multi-location trials across different centers to identify the best one for release as a commercial composite variety.

Key words

Polycross breeding, half sib, palmarosa, composite hybrids, essential oil yield.

INTRODUCTION

Palmarosa (*Cymbopogon martinii* [Roxb.] Wats. Var. motia Burk., family, Poaceae), is an important aromatic crop that is commercially cultivated in India for the essential oil that is rich in geraniol, geranyl acetate and linalool. It has many uses in soap, perfumery, and cosmetic industries due to its aroma like rose (Sahu *et al.*, 2000; Verma *et al.*, 2010). It possesses many antifungal and antibacterial medicinal properties. Considering its economic importance, agriculture research has been initiated in all aspects including its improvement. In India palmarosa has the advantage of being cultivated both

through seed and vegetative propagation. This gives two options to the breeders to improve the crop through breeding approaches that will be suitable for seeds as well as vegetative propagated species. The improvement through seed propagation method in palmarosa needs special attention as the species is highly cross pollinated and its genetic behaviour is distinctly different from other seed propagated crops. There are several breeding methods available for cross pollinated crops and among them population improvement methods are often used for grass species (Rosulj *et al.*, 2002; Dudley and Lamert,

1992). The development of composite/synthetic varieties for yield improvement is also successfully demonstrated in many crop species like maize, (Misevic *et al.*, 1989; Sprague and Brimhall, 1950 and Sprague *et al.*, 1952). Any improved variety multiplied through seeds in cross pollinated species like palmarosa needs identification of desirable combination of donor parents. In this direction the present investigation attempted to follow the mass selection procedure with slight modifications to improve the herbage and oil yield in palmarosa (Smitha and Manivel, 2021). Hence, in this paper, an attempt was made to raise the best fifteen identified clones in polycross nursery with the following objectives (i) to raise the best identified clones in a polycross nursery and to allow (for random mating) if the term is appropriate, it can be included with all possible combinations (ii) to evaluate the performance of composite hybrids along with the check (iii) to identify the best performing polycross hybrids for yield and essential oil for release as a commercial synthetic variety and (iv) to identify the best performing polycross progeny composite

hybrid for yield and essential oil content, and for releasing as a commercial synthetic variety after assessing its stable performance under multi-location trials.

MATERIALS AND METHODS

Fifteen best palmarosa clones were identified from two years screening of 1200 plants of seven germplasm groups for different growth, yield and quality parameters (Smitha and Manivel, 2021) and formed the base material for this study. The slips of fifteen clones were planted in the polycross nursery during June 2011 in isolation at a spacing of 1 × 1 m at ICAR-Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat, India (22° 35' N; 72° 55' E). The plot size was 150 m² i.e., one hundred and fifty plants per polycross nursery. The climate of the location comprises of mild winter and warm dry. The clones were planted in ten replications and randomized in such a way that each clone will get an equal chance of out-crossing. The experimental details are depicted in schematic diagram (Fig. 1).

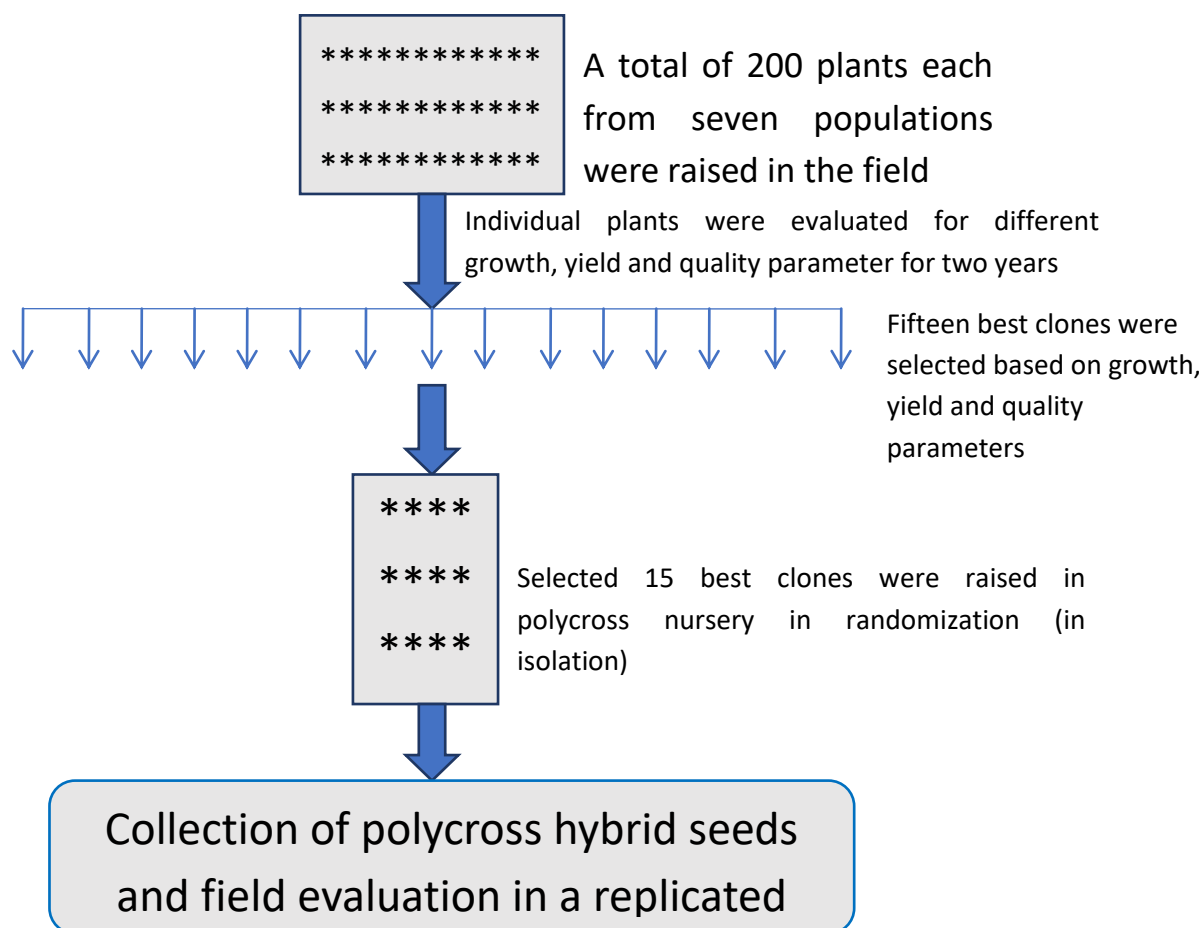


Fig. 1. Schematic representation of modified mass selection in palmarosa

The composite hybrid seeds of palmarosa were collected separately from each clone from the polycross nursery. The fresh seeds collected from the polycross nursery were raised in the nursery beds during June 2012 with the onset of monsoon. The field evaluation of fifteen polycross hybrids was done individually and in combination along with a check, Trishna, a commercially released variety. The evaluation experiment comprised of 17 treatments consisting of fifteen hybrid seeds DCMH-1 to DCMH-15 as T_1 to T_{15} , while T_{16} comprised of a mixture of all 15 hybrid seeds mixed in equal proportions and T_{17} is a variety Trishna which was used as a check. All the treatments were replicated thrice in RBD. All the hybrids were raised in nursery and seedlings were transplanted in the main field in Boriavi farm during June 2012 with a spacing of 1×1 m. Each plot is of size 30 m^2 i.e., thirty plants/plot.

The crop was harvested at full flowering stage after three months of planting by cutting the individual clump at 15 cm above the ground and allowed the remaining clump for ratooning. Subsequent three harvests were also taken at once in every three months interval. The fresh weight of whole herb was recorded using electronic balance, and a sample of herb was dried at 105°C for ~ 72 h and dry matter content was recorded. At each harvest, observations of five plants per plot was recorded with respect to growth parameters such as plant height, the number of tillers, yield dry weight, essential oil content and essential oil yield. The naturally crossed hybrid seeds from plants of each individual seeds from plants of each individual clones were harvested separately.

The shade dried aerial parts from each plant including leaves; stem and inflorescence (200g each) were hydro-distilled in Clevenger type apparatus for 3hrs. The distillate was extracted with diethyl ether, the ethrel layer was dried over anhydrous sodium sulphate and ether distilled off on gently heated water bath. The oil content per plant was expressed as % w/w on dry weight basis. The essential oil yield per plant was estimated by multiplying oil content per plant and its dry weight and expressed as ml/plant.

During evaluation, the following variables were documented: plant height, the number of tillers, dry weight, essential oil content and essential oil yield per plant. Collected data were subjected to two-way ('genotype \times season') ANOVA related to the experiment.

The analysis of variance was done in a randomized block design for various observations recorded by using statistical software SAS 9.2 (Anon, 2008). Comparison was made between different clones for growth, yield and quality parameters over period of two years. The results were presented at 5% level of significance. The critical difference (CD) values were calculated to compare the various treatment means.

RESULTS AND DISCUSSION

George Acquaah (2012) reported that the polycross method of breeding is most suited to species that are obligate cross pollinated and can be propagated vegetatively by providing an equal opportunity for each entry to be crossed with every other entry. Palmarosa being a cross pollinated crop in the family Poaceae can also be propagated through clonally and is most suitable for polycross breeding unlike crops like Little millet (*Panicum sumatrense*) wherein modified crossing method for artificial hybridization is commonly used (Nandini *et al.*, 2019). Considering this in the present study the performance of parental clones of palmarosa in polycross nursery was studied over two years (four harvests) for five traits (**Table 1**). Significant differences were observed among the clones for all the five characters studied and that indicated the existences of considerable variability among the polycross progenies. The plant height ranged from 137.8cm to 188.6cm (DCM 12) with a mean of 157.67, whereas the number of tillers ranged from 192.6 to 345.2 and three clones recorded >320 tillers. For dry herbage yield the mean was 0.85kg/plant; seven clones had lower than general mean and remaining clones had higher values. Seven clones had higher essential oil content and oil yield than the general mean (**Table 1**).

In general, the values for each trait studied in polycross clones (**Table 1**) were higher than the values obtained in corresponding values in its mother seedlings clones (Smitha and Manivel, 2021) and their composite hybrid progeny. This is because the polycross clones are genetically the combination of different superior mother clones selected based on their best performance and allowed them to mate freely and randomly. It gives a clear indication that there is an ample scope to develop superior composite varieties in palmarosa using polycross breeding. Total oil yield in palmarosa is directly correlated on herbage yield and oil content (Smitha and Manivel, 2021). Hence, as expected, the higher oil yielding clones had higher dry herbage yield and besides they had either higher tiller number or plant height or both. It means, for getting higher oil yield both high dry herbage yield and oil content are found to be the essential characters. It was clear that the clone DCM 14 had the higher oil content of 2.5 per cent (on dry weight basis) but its oil yield was only 11.75 g/plant and its dry herbage yield was only 0.47kg/plot. Whereas, the clone DCM2 had the highest oil yield (23.23g/plant) which was due to high oil content (2.3%) and high herbage yield (1.01g/plant). This gives clear indication that for developing high oil yielding palmarosa varieties/hybrids/polycross hybrids/varieties the breeder should go for simultaneous selection for high herbage yield along with high oil content besides other yield contributing traits.

In an open pollinated population of crops like palmarosa, each individual plant will get an equal opportunity to cross with each other as each individual is genetically different

Table 1. Growth, yield, and quality parameters of selected clones in polycross nursery (Average of two years)

Clone	Plant height (cm)	Number of branches	Dry weight (kg/plant)	Oil content (%)	Oil yield (g/plant)
DCM – 1	144.2	292.8	0.81	1.7	13.77
DCM – 2	157.0	321.0	1.01	2.30	23.23
DCM – 3	162.2	294.4	0.73	1.70	12.41
DCM – 4	151.2	338.4	1.00	2.30	23.00
DCM – 5	148.8	345.2	0.85	1.30	11.05
DCM – 6	151.2	297.2	1.02	2.1	21.42
DCM – 7	181.8	251.2	0.76	1.7	12.92
DCM – 8	170.4	271.2	1.18	1.70	20.06
DCM – 9	145.3	207.7	1.05	2.20	23.10
DCM – 10	155.4	233.4	0.98	1.90	18.62
DCM – 11	157.0	192.6	0.71	1.90	13.49
DCM – 12	188.6	259.4	1.04	2.23	23.19
DCM – 13	169.2	264.8	0.75	2.05	15.38
DCM – 14	137.8	260.8	0.47	2.50	11.75
DCM – 15	145.2	266.8	0.33	1.70	5.61
Mean	157.67	273.13	0.85	1.95	16.56

from one other i.e., heterozygous. In such situation, the open pollination with pollen from different individuals will produce different gene recombinations. Some may combine well and produce high heterotic vigour for different traits of interest and others may not. If we compare the performance of offspring and the individual clones, it is expected that it will follow normal distribution for each trait. However, the mean may skew towards either positive or negative side. It is again dependent on the gene combination of ovule parent and pollen parent. In a population, certain clones may produce good performing progenies when crossed with any pollen parent, due to its good general combining ability. Whereas, if the same clone is producing a better performing offspring only when crossed with specific clones/parents and not with other clones, it is because of its specific combining ability. Both the types of clones are important for plant breeders as they have their own advantages and could be used as by the need of the breeders. Aastveit and Aastveit (1990) developed a program for progeny testing and the selection in base population of perennial forage grasses with many randomly selected parental clones and a fixed number of clones from each of the half-sib families derived from the mother genotypes are grown simultaneously. The clones selected within superior families can be further cloned, placed in a polycross field, and the new half sib families can be tried in field trials at different locations for further selection.

Open pollinated hybrid seeds obtained from all the fifteen parental clones from the polycross nursery was evaluated in a replicated trial for five traits (plant height, the number of tillers, dry weight, essential oil content and essential oil

yield (**Table 2**). In the present study, all the fifteen clones raised in polycross nursery were tested for its general combining ability. Studying the performance of hybrids seedlings obtained from the fifteen clones for different traits revealed that two hybrids DCMH 8 and DCMH 10 had higher dry herbage yield than the check variety Trishna (0.68kg/plant) even though they were statistically on par. These hybrids recorded higher oil yield per plant also than the check. The varying level of performance by 15 polycross progenies is expected as they are differ in their heterozygosity for different traits as a result of random matting between the selected parental clones in the polycross nursery. The breeder must identify the best one and, in that line, from the present study the two hybrids DCMH 8 and DCMH 10 were identified for further evaluation.

Significant differences were observed among fifteen composite hybrids during the first and second harvest, whereas it was non-significant among the third and fourth harvest. It indicates that the plant height increases significantly in the different hybrids whereas, it was non-significant when a repeated cutting was given. As observed in the parental clonal generation (Smitha and Manivel, 2021), in the hybrid progeny also the maximum plant height was observed in the third harvest. The plant height gradually increased in the second harvest and reached the maximum in the third harvest. The mean height of all the four harvests also showed significant differences among the 15 composite hybrids. Among the 15 composite hybrids, DCMH 12 and DCMH 8 were significantly taller than the commercial variety (Trishna). Whereas, all the other hybrids (except DCMH 3), were

significantly on par for height with the check variety. It may be concluded that not only the season of harvest, but also the age of the plant determines the maximum expression of this trait. From the study on comparison of plant height with the parental clones (**Table 1**) and the composite hybrids (**Table 2**) it is observed that the hybrid progenies were taller than their respective parental clones in all the 15 hybrids. This may be due to the heterotic expression of the progenies that were produced from the best performing clones for different traits from the polycross nurseries.

The mean plant height of different harvests over 15 hybrids (**Table 2**) were resulted in plant height of 159 cm in the first harvest which increased to 179 cm in the second harvest to 220 cm in the third harvest and subsequently reduced to 187.8 cm at the fourth harvest. However, the same trend was not observed for the number of tillers but there was an increase in the number of tillers from the first to second harvest which further increased in the third and fourth harvests. Out of 15 composite hybrids, ten hybrids recorded maximum number of tillers during the second harvest and remaining five hybrids in the fourth harvest.

The hybrids, DCMH 10, DCMH 7 and DCMH 14 recorded the maximum number of tillers per plant. However, none of the hybrids had a significantly higher tiller number than the check variety Trishna. Ten hybrids recorded a greater number of tillers which were on par with check variety. The performance of parental clones in the polycross nursery v/s the respective composite hybrid progenies in the evaluation trial indicated that all the parental clones had higher values than the progenies (**Table 1 & 2**) and it is expected as the clones in the polycross nurseries were the best performing clones selected based on their the number of tillers, dry weight and other parameters. At the same time the combinations of genes for the number of tillers in the progenies (heterozygous and heterogeneous) were not adequate to outperform for the number of tillers in the present study.

The dry weight ranged from 0.39-0.86kg/plant as compared to 0.68kg in I Trishna. Two hybrids viz., DCMH 8 and DCMH 10 recorded significantly higher dry weight yield than the check variety. Whereas all the other hybrids (except DCMH3 and DCMH13) had significantly on par dry weight with the check variety. For this character also

Table 2. Performance of composite hybrid seeds (half sib) in different harvests

Polycross hybrid	Plant height (cm)					Number of tillers per plant				
	I Harvest (Oct 2013)	II Harvest (Jan 2014)	III Harvest (April 2014)	IV Harvest (July 2014)	Mean	I Harvest (Oct 2013)	II Harvest (Jan 2014)	III Harvest (April 2014)	IV Harvest (July 2014)	Mean
T ₁ – DCMH 1	160.13	185.31	222.47	196.93	191.21	71.73	138.55	130.81	122.07	115.79
T ₂ – DCMH 2	150.61	151.83	213.20	190.40	176.51	96.29	111.31	100.58	105.87	103.51
T ₃ – DCMH 3	136.67	133.37	201.92	181.47	163.36	70.50	62.87	70.05	101.27	76.17
T ₄ – DCMH 4	154.07	189.81	235.59	196.27	193.93	66.87	145.53	126.56	126.47	116.36
T ₅ – DCMH 5	146.87	166.29	214.36	196.60	181.03	71.67	110.26	98.14	132.33	103.10
T ₆ – DCMH 6	172.67	198.51	218.92	202.07	198.04	89.80	156.58	112.20	124.80	120.84
T ₇ – DCMH 7	169.93	192.13	202.33	205.87	192.57	81.40	156.14	90.31	163.60	122.86
T ₈ – DCMH 8	176.13	197.89	233.05	198.00	201.27	76.47	146.60	131.53	123.58	119.54
T ₉ – DCMH 9	156.47	183.88	224.33	190.27	188.74	87.00	138.71	125.30	128.67	119.92
T ₁₀ – DCMH 10	174.40	205.69	218.33	198.13	199.14	102.93	180.61	111.97	145.53	135.26
T ₁₁ – DCMH 11	168.47	197.35	226.42	199.67	197.97	67.20	139.52	139.86	122.67	117.31
T ₁₂ – DCMH 12	176.27	188.03	241.89	207.27	203.36	74.27	130.22	104.83	141.33	112.66
T ₁₃ – DCMH 13	144.92	131.57	211.95	191.67	170.02	70.25	57.92	98.11	98.33	81.15
T ₁₄ – DCMH 14	154.13	176.27	208.47	182.67	180.38	110.60	149.31	110.11	121.80	122.96
T ₁₅ – DCMH 15	139.73	180.72	222.25	179.40	180.53	60.20	128.37	109.92	117.53	104.00
T ₁₆ – Mix	159.20	170.37	223.09	203.47	189.03	63.33	87.24	113.78	139.60	100.99
T ₁₇ – Trishna	160.07	187.60	200.56	188.40	184.16	95.67	152.29	131.67	153.60	133.31
SEm±	7.50	11.65	11.81	7.22	5.28	15.26	16.22	13.42	15.00	8.85
C.D. at 5%	21.60	33.58	NS	NS	15.10	NS	46.74	NS	NS	25.30

Table 2. continued. Performance of composite hybrid seeds (half sib) in different harvests

Polycross hybrid	Dry herbage yield (kg/ plant)					Oil content (%)			Oil yield (ml/plant)		
	I Harvest (Oct 2013)	II Harvest (Jan 2014)	III Harvest (April 2014)	IV Harvest (July 2014)	Mean	I Harvest (Oct 2013)	II Harvest (Jan 2014)	Mean	I Harvest (Oct 2013)	II Harvest (Jan 2014)	Mean
T ₁ – DCMH 1	0.50	0.83	0.75	0.58	0.66	2.42	1.63	2.03	11.78	13.17	12.48
T ₂ – DCMH 2	0.45	0.66	0.64	0.26	0.50	2.50	1.24	1.87	11.26	8.44	9.85
T ₃ – DCMH 3	0.30	0.33	0.45	0.34	0.35	1.67	1.84	1.76	4.96	6.19	5.58
T ₄ – DCMH 4	0.33	0.80	0.76	0.58	0.62	2.17	1.20	1.69	7.50	9.64	8.57
T ₅ – DCMH 5	0.29	0.63	0.73	0.46	0.53	2.30	1.17	1.74	6.67	7.57	7.12
T ₆ – DCMH 6	0.38	1.16	0.59	0.55	0.67	2.48	1.39	1.94	9.13	14.91	12.02
T ₇ – DCMH 7	0.38	1.00	0.79	0.46	0.66	2.52	1.41	1.96	9.69	13.93	11.81
T ₈ – DCMH 8	1.00	1.14	0.76	0.51	0.86	2.03	1.12	1.58	19.40	12.77	16.08
T ₉ – DCMH 9	0.42	0.93	0.60	0.41	0.59	2.40	1.52	1.96	10.05	14.35	12.20
T ₁₀ – DCMH 10	0.50	1.20	0.74	0.86	0.83	2.35	1.57	1.96	11.27	17.95	14.61
T ₁₁ – DCMH 11	0.34	0.80	0.76	0.49	0.59	2.63	1.41	2.02	8.83	10.95	9.89
T ₁₂ – DCMH 12	0.45	0.79	0.77	0.52	0.63	2.37	1.49	1.93	10.23	12.40	11.32
T ₁₃ – DCMH 13	0.29	0.36	0.62	0.28	0.39	1.92	1.36	1.64	5.56	4.94	5.25
T ₁₄ – DCMH 14	0.46	0.85	0.67	0.55	0.63	2.85	1.50	2.18	13.66	13.11	13.39
T ₁₅ – DCMH 15	0.26	0.79	0.66	0.50	0.55	2.12	1.28	1.70	5.62	10.22	7.92
T ₁₆ – Mix	0.30	0.59	0.68	0.60	0.54	1.80	1.00	1.40	5.47	5.82	5.64
SEm±	0.16	0.12	0.09	0.11	0.07	0.14	0.19	0.17	3.19	2.22	2.71
C.D. at 5%	NS	0.34	NS	NS	0.19	0.41	NS	0.41	NS	6.40	6.40

the maximum yield was recorded in different harvests by different genotypes. Most of the hybrids (twelve) had higher yield in the second harvest; the remaining three hybrids had higher values in the third harvest. The performance of clones was compared to its original parental population (Smitha and Manivel, 2021) which shows that the parental clones attain harvest at 8 months after transplanting in the main field. Whereas, in polycross hybrids, the first harvest was obtained immediately after three months of planting and subsequent harvests were also obtained at an interval of every three months. Similar trend for dry matter was observed when compared to parental clones with polycross hybrids for this character also. Two hybrids DCMH 8 and DCMH 10 had more than 22 per cent higher dry yield than the commercial variety Trishna. Interestingly, these two hybrids produced maximum dry herbage yield in the first harvest itself. This can be used as one of the selection criteria for developing high yielding varieties/composite hybrids in palmarosa. Similarly, Wagoner (1995) evaluated 400 progenies of wheat grass (*Thinopyrum intermedium*) in polycross nursery and got approximately 25 per cent higher grain yield per plant in the 14 selections compared to population mean. Further Kolliker *et al.* (2005) in polycross breeding of perennial ryegrass (*Lolium perenne* L.) reported that the diversity

within first generation synthetic progenies (Syn1) was proportional to the diversity within the respective parental polycrosses. They also found that progenies from wide polycrosses are constantly giving higher yield compared to progenies from narrow polycrosses.

The oil content was estimated in two harvests only. Among the 15 hybrids eight hybrids had higher oil content than the check variety Trishna (1.88%). Though all were statistically on par, significant differences were observed among the hybrids. The oil content in the hybrids was the highest during the first harvest (October 2013) as compared to the second harvest which was done during January 2014. It indicated that the essential oil content was higher in the rainy season crop than the winter season crop as reported by Smitha and Rana (2015). The reduction was almost half in most of the lines. The mean oil content ranged from 1.58 (DCMH 8) to 2.18 per cent (DCMH 14).

The oil yield was recorded only in two harvests i.e., 1st and 2nd. The mean oil yield was higher in the second harvest than the first harvest; it might be due to increased herbage yield. Even though the overall mean of the second harvest is double than the first harvest, it was not reflected in

oil yield. It was because of the oil content in the first harvest was very high than the second harvest. It gives an indication that for high oil content, the rainy season is better than the winter season, even though it favors for higher herbage yield in palmarosa. Similar results on influence of seasons on essential oil yield was reported by Smitha and Rana (2015) in Palmarosa and Smitha and Vandana Tripathy (2016) in different *Ocimum* species. There was significant difference among the 15 hybrids for oil yield. In the present study, all the seven hybrids had numerically higher values than the check variety Trishna, but were statistically on par. Among the hybrids DCMH3 had the lowest yield of 5.58 g/plant and DCMH8 had the highest (16.08 g/plant). Seven hybrids had higher value than Trishna but were statistically on par. DCMH8 had 37.79 per cent higher oil yield than check variety, as expected as this hybrid had a high dry herbage yield in comparison to Trishna and can be promoted further.

Palmarosa is a cross pollinated crop which can be improved through population improvement using appropriate breeding method. In the present investigation, the modified mass selection followed by identification and evaluation of polycross composite hybrids for different morphological, yield and essential oil traits resulted in identification of superior hybrids viz., DCMH1, DCMH6, DCMH8, DCMH10, and DCMH14 for further exploitation in the commercial scale.

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