Genetic improvement of palmarosa (Cymbopogon martinii var. motia) for herbage and essential oil yield through polycross method of breeding

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Abstract
Palmarosa (Cymbopogon martinii var. motia) is an important essential oil-bearing plant that is used worldwide in the soap, perfumery and cosmetic industries. It is a highly cross-pollinated crop and can be propagated sexually through seeds or clonally by slips. The main objective of the present investigation is to collect populations of palmarosa from different parts of India, to analyse the variability present within and between population, to study the correlation of different traits and to select the best performing clones in terms of growth, herbage and essential oil yield for raising the best performing clones in polycross nursery. The seeds of seven populations/varieties were collected from various parts of India and two hundred seedlings of each population were raised and transplanted to the field at the ICAR-Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat, India. Observations on individual plants performance viz., plant height, number of tillers, dry herbage yield and essential oil yield were analysed for five harvests which were taken over a period of two years. For plant height, a maximum frequency of 32 per cent was recorded from 141 to150 cm in PRC-1, whereas, for the number of tillers it was 31.88 per cent from the Bengaluru collection that was between 81 and 110 tillers. Fresh and dry herbage yield showed a significant positive correlation with plant height, the number of tillers and oil yield. The correlation between dry herbage yield and the number of tillers was maximum (0.761), followed by dry herbage yield and essential oil yield (0.563) and the number of tillers and oil yield (0.417). Interestingly, a significant correlation of oil yield with plant height and number of tillers gives an indication that the improvement of the traits will not only increase the dry herbage yield but also increase the essential oil yield. Based on the performance of individual clones out of 642 clones evaluated, the best fifteen clones were selected based on the dry herbage and essential oil yield and named as DCM-1 to DCM-15. These clones were further utilized for raising polycross nursery.

Key words
Palmarosa, morphological characters, herbage yield, essential oil, polycross hybrids.

INTRODUCTION
Palmarosa (Cymbopogon martinii [Roxb.] Wats. var. motia Burk.), also known as Russia/Rosha grass, Barbed wire grass, Fever grass, Silky heads, Hierba Luisa etc. yields aromatic oil of commercial value. It is a cash crop in India (Kavya Dashora and Gosavi, 2013) that can adapt to varied edapho-climatic conditions and occurs generally in the tropics and subtropics (Sangwan et al., 2001). The flowering tops and leaves of the plant have essential oil which is rich in geraniol, geranyl acetate and linalool (Smitha and Rana, 2015). It is commonly used in the perfumery, soap, and cosmetic industries because its aroma is similar to that of rose (Sahu et al., 2000; Verma et al., 2010). Geraniol in palmarosa oil provides antifungal and antibacterial properties (Bard et al., 1988). Palmarosa is native to India and naturally grows in the forests of Andhra Pradesh, Karnataka, Tamil Nadu, Maharashtra, Madhya Pradesh, and Uttar Pradesh. Apart from India, it is cultivated in Brazil, Bulgaria, Paraguay, Madagascar,
Guatemala, and Indonesia (Sahu et al., 2000; Singh and Kumar, 2000; Verma et al., 2010). It can be cultivated in problematic areas with soil sodicity with three to four times more profit than the cultivation of rice and wheat (Prasad and Patra, 2004; Gogi and Deka, 2015). India is the key producer and supplier of Palmarosa oil to the global market which is being exported to about 19 countries, including the United States, France, and the Philippines (Anonymous, 2010). Earlier, India had its monopoly in palmarosa oil production, which has now facing competition with Bulgaria, Guatemala and Brazil.

Initially, the palmarosa plants from the wild were used for the extraction of oil, but they had lower oil yield and inferior quality due to the inherited genetic heterogeneity, out crossing and ecological evolution. The need for quality palmarosa oil production and its increased demand in national and international market has restricted the distillation from wild sources and the distillers demanded good seeds, which gradually attracted the attention of scientific workers and plant breeders to investigate the ways to improve this crop.

Palmarosa with diploid chromosome number 2n=20 is a perennial grass, dark green colour leaves, fibrous shallow roots, culms erect, 1 to 2 m height, nodes swollen and glabrous. It has 25 to 35 per cent hermaphrodite flowers and the rest being stamineate florets in the inflorescence. Pollen fertility varied from 84 to 96 per cent in staminate and hermaphrodite flowers. Seed setting was 15 per cent in self, 50 to 64 per cent in open pollination confirms that it is a highly cross-pollinated species (Srivastav and Srivastav, 1988). Because of this reason, a lot of variations for different quantitative and qualitative traits have been reported.

As palmarosa can be multiplied through seeds as well as clones, clones vs seed progeny selections have been reported by several workers. Sahu et al. (2000) evaluated 85 clonal lines and selected 19 clones and compared them with the selections made at various research organizations in India. RRL(B)-77 and IW-31245 were found to be suitable for various environmental conditions. PRC-1 is a comparatively early maturing strain that could be further improved for its oil yield potential. Selections RRL(B)-77, IW-31245, PRC-1, HR-49, CI-77-1, IW-3631, ODP-1, ODP-2, IW-4498 and C-3 are now being cultivated in different parts of India. The stabilized palmarosa clonal selections had recorded narrow ranges of variation for most of the traits. But the out crossing among the selected genotypes could be used for creating further variation and population improvement programmes. A high yielding polycross variety Trishna was released from CSIR-CIMAP, Lucknow, through mass selection (Sharma et al., 1987).

Mass selection is the oldest and simplest form of recurrent selection breeding method that involves cyclic breeding procedure intended to improve traits of populations especially in cross pollinated crops like palmarosa. However, the effectiveness of this method depends on the heritability of the traits (Gardner, 1961). The usefulness of this method of breeding has been related to yield, earliness, ear height and ear length in corn (Weyhrich et al., 1998; Burton and Widstrom, 2001). Many success stories have been reported in field crops like maize (Rosulj et al., 2002; Dudley and Lamert, 1992; Misevic et al., 1989; Sprague and Brimmall, 1950 and Sprague et al., 1952) utilised a variation of mass selection and reported increased oil content per cycle of selection for five years in maize. In this direction, in the present investigation, certain modifications were made in the mass selection procedure and efforts were made to improve the palmarosa for herbage and oil yield.

**MATERIAL AND METHODS**

The seeds of seven populations were collected from different parts of India and used in the study. These populations and their sites of collections were: V1 = Anand, Gujarat, India, V2 = Udaipur, CIMAP, Lucknow, V3 = Amravati, Maharashtra, V4 = Vadtal, Gujarat, V5 = Bengaluru and V6 = Indore, Madhya Pradesh. At each site, random samples of 100 g seeds were collected during summer, 2009. From each selection, seedlings were raised in the nursery beds during the monsoon (June 2009). When the seedlings were one month old, randomly selected 200 seedlings each individual selections were transplanted into the main field at a spacing of 60 × 60 cm in separate plots during July 2009 at the ICAR-Directorate of Medicinal and Aromatic Plants Research (DMAPR), Anand, Gujarat, India. A considerable effort was made to maintain the plant stand by regular gap filling at the initial stages. However, many plants could not able to withstand due to repeated harvesting/other uncontrollable reasons and at the end of the second year, the final plant stands in each selection was; V1=131, V2= 33, V3= 43, V4=103, V5=135, V6= 138 and V7=59. At the time of planting and after each harvesting farmyard manure was applied @ 20 t/ha. All the plant production and protection measures recommended to have a healthy crop were followed.

During the initial period of establishment, the growth was very slow and was able to take up the first harvest at eight months after planting at the full flowering stage. The subsequent harvests were done at a regular interval of three months i.e., 11, 14, 17 and 20 months after planting depending upon the growth of the plant at different seasons. Harvesting of the crop was done at the full flowering stage by cutting the individual clump 15 cm above the ground and allowed the remaining clump for ratooning. In total, five harvests were taken over a period of two years (2009-10 and 2010-11). At each harvest, observations on individual plants performance plant height (cm) and the number of tillers, dry herbage (kg/ plant) and essential oil yield (g/plant) were recorded.

The shade dried aerial parts from each plant including leaves, stem and inflorescence (200 g each) were
Table 1. Mean performance of varieties (V) for different morphological traits and oil yield at different months after planting (MAP)

| Population / Variety | Harvest | Average of five harvests |
|----------------------|---------|--------------------------|
|                      | Mean    | Range        | Mode  | Median | Variance | Skewness |
| V1                   | 179.28  | 171.4        | 169.8 | 152.79 | 18.4     | -0.18    |
| V2                   | 161.41  | 147.0        | 170.2 | 149.05 | 17.3     | -0.41    |
| V3                   | 152.79  | 160.08       | 160.8 | 146.41 | 19.4     | -0.55    |
| V4                   | 145.15  | 139.47       | 153.0 | 152.57 | 22.5     | 0.03     |
| V5                   | 150.24  | 163.94       | 151.6 | 159.72 | 25.7     | -0.87    |
| V6                   | 169.84  | 175.49       | 169.0 | 165.19 | 31.6     | 0.13     |
| Mean                 | 157.12  | 162.46       | 156.6 | 147.48 | 58.6     | 0.33     |

Number of tillers per plant

| Population / Variety | Mean  |
|----------------------|-------|
| V1                   | 136.2 |
| V2                   | 131.0 |
| V3                   | 112.8 |
| V4                   | 133.2 |
| V5                   | 126.1 |
| V6                   | 121.7 |
| V7                   | 127.3 |
| Mean                 | 126.9 |

Dry herbage yield (kg/plant)

| Population / Variety | Mean |
|----------------------|------|
| V1                   | 22.6 |
| V2                   | 22.6 |
| V3                   | 22.6 |
| V4                   | 22.6 |
| V5                   | 22.6 |
| V6                   | 22.6 |
| V7                   | 22.6 |
| Mean                 | 22.6 |

Essential oil yield (g/plant)

| Population / Variety | Mean |
|----------------------|------|
| V1                   | 0.51 |
| V2                   | 0.51 |
| V3                   | 0.51 |
| V4                   | 0.51 |
| V5                   | 0.51 |
| V6                   | 0.51 |
| V7                   | 0.51 |
| Mean                 | 0.51 |

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RESULTS AND DISCUSSION
A total of 1200 clones from 7 populations were planted and evaluated for four traits viz., plant height, the number of tillers per plant, dry herbage yield, and essential oil yield per plant. The mean values for five harvest made at different intervals are presented in Table 1. Variation was observed within and between germplasm for all the traits (Fig.1). Similar significant variations have been already reported in this crop by various workers (Sahu et al., 2000; Verma et al., 2010). Such significant variations are highly desirable to the plant breeders to develop high yielding varieties. The variance was the highest for the number of tillers followed by plant height, essential oil and dry weight. The variability among the plants of each germplasm and between the germplasm is expected, as the plants were raised using seeds as planting material, that are genetically heterogeneous and heterozygous in nature as these seeds were obtained from cross pollination between heterozygous and heterogeneous parent plants. Plant height ranged from 104 to 202.4 cm, the shortest plant was from V4 and the tallest plant was from V1. The minimum number of tillers (22.6 per plant) was recorded in a plant of V5, whereas, the maximum of 290 tillers per plant in a plant of V1. Amongst the seven germplasm accessions, V6 had the plant with the lowest herbage yield (0.04 kg/plant) and a maximum of 1.14 kg/plant was recorded in V1 and V2, respectively. The essential oil yield ranged from 0.05 (V7) to 9.08 ml/plant (V1).

The variance for plant height was ranged from 179.42 to 274.26 (V5), for tillers. It was from 1408.96 (V5) to 2579.45 (V3), for dry herbage yield 0.012 (V6) to 0.077 (V2), whereas, for oil yield, it was between 4.15 (V5) and 13.04 (V3) (Table 1). The mean modal class was 158.46 for plant height and the minimum was from V4 (137.40) and the maximum was from V1 (171.4). Similar variation was

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Fig. 1. Variation within and between germplasm for morphological traits and oil yield in palmarosa (mean over five harvests done over a period of two years)
observed for the other three traits also. For skewness, all germplasm (except V7) had negative values. On the other hand, all had positive values (except V3) for the number of tillers per plant. The maximum skewness value of 3.81 was recorded in V6 for oil yield and the minimum value of -0.71 for plant height in V5.

For plant height, the maximum frequency of 32 per cent was recorded between 141 and 150 cm in V3, whereas, for the number of tillers it was 31.88 per cent in V6 that was between 81 and 110 tillers (Fig.2). For dry herbage yield, the maximum per plant i.e., 66.67 per cent was recorded under 0.10-0.25 kg/plant by V6. However, V1 had a maximum percentage of plants (55%) under 0.1-0.3 g/plant for essential oil yield. From the present study, there exist variations between the germplasm accessions for all the four traits studied. Out of 7 collections, V3 i.e. the collection from CIMAP Lucknow showed more desirable plants with higher values for the traits studied. For most of the germplasm, the maximum expression for all the traits was obtained in the third harvest i.e., 14 months after planting that coincides with the September month. The trend was gradually increasing in the beginning till 3rd harvest and gradually decreasing thereafter till 5th harvest. It indicates that initially, the repeated harvest increases the plant height and the number of tillers, the dry herbage yield. After that rationing ability of the mother plant reduced as evident from reduction in all these parameters. This can very well be correlated with the weather parameters high rainfall, high relative humidity, lower minimum and maximum temperature and low evapo-transpiration as compared to the harvest done in other seasons and the third harvest was immediately after the rainy season (October). It indicated that the weather factors played a major role in the expression of these traits (Smitha and Rana, 2015).

The association between the four traits studied at each harvest is given in Table 2. The correlation values are changing at each harvest within and between traits. In every harvest, the dry weight of the plant was significantly correlated with plant height, the number of tillers and fresh herbage yield. Only in the first harvest number of tillers had a significant correlation with plant height (0.3), indicating that in the initial stage of seedling growth these traits depend on each other and later stage they are not significantly dependent on each other. The correlation between dry herbage yield and the number of tillers was maximum (0.761), followed by dry herbage yield and oil yield (0.563) and the number of tillers and oil yield (0.417) and were significant. Plant height had a positive
and significant correlation with dry herbage yield but non-significant and low values with the number of tillers and oil yield. For plant height, the correlation between different days after harvesting is non-significant (except between harvest at 8 and 11 months after planting) and values ranged from 0.03 to 0.38. For the number of tillers, the correlation between different dates of the harvest was ranged from 0.35 to 0.53 and significant (Table 3). The number of tillers per plants is the most important traits followed by plant height and hence, considered as selection criteria in the palmarosa breeding programme. The results are in conformity with Sabesan et al. (2009) in rice wherein grain yield per plant was recorded positive significant correlation with plant height and the number of productive tillers per plant at both genotypic and phenotypic levels. Similarly, in bread wheat genotypes, a positive genotypic association for grain yield was found with chlorophyll content (0.601), plant height, (0.331), spike length (0.301) the number of spikes per plant (0.883), thousand kernel weight (0.468) and harvest index (0.561) and hence, these traits can be considered while developing high yielding genotypes (Fellahi et al., 2013).

As palmarosa is a perennial crop, repeated harvests are done from ratoon crops which are equally profitable as the main crop (Smitha et al., 2015; Chauhan et al., 2017). In the present study, the herbage yield significantly increased from the first harvest (8 months after planting) to subsequent harvest at every three months interval up to 14 months after planting, and thereafter it reduced. This is very well justified from the correlation values obtained between different harvesting intervals. It is clear in the four harvests done at regular interval the yield of one harvest highly depends on its yield of the previous harvest. Initially, the plant height was significantly contributed for dry herbage yield up to the second harvest (11 months after planting), later the contribution of plant height is non-significant and the number of tillers took over the significant contribution. But, after 17 months after planting, as both plant height and the number of tillers came down, the herbage yield also automatically started coming down. The reduction may be due to the age of the plants. In commercial cultivation of palmarosa, farmers grow the crop till four to five years depending on the management practices followed and then it is advised to go for a new planting (Suresh et al., 2014).

Table 2. Pearson's correlation coefficients for growth, yield and oil characteristics of palmarosa germplasm (average of four harvests)

|                | Plant height | Number of tillers | Dry weight | Oil yield |
|----------------|--------------|-------------------|------------|-----------|
| Plant Height   | 1.00         |                   |            |           |
| Number of tillers | 0.012        | 1.00              |            |           |
| Dry weight     | 0.078        | 0.761*            | 1.000      |           |
| Oil yield      | -0.01374     | 0.417*            | 0.563*     | 1.000     |

Table 3. Pearson's correlation coefficients for various growth and yield characters at different months after planting (MAP) in palmarosa germplasm

|                | Plant height | Number of tillers | Dry Weight |
|----------------|--------------|-------------------|------------|
| **8 MAP**      | 1.00         |                   |            |
| **11 MAP**     | 0.38*        | 1.00              |            |
| **14 MAP**     | 0.08         | 0.03              | 1.00       |
| **17 MAP**     | 0.02         | 0.09              | 0.12       | 1.00     |
| **8 MAP**      | 0.30*        | 0.04              | -0.21*     | 1.00     |
| Number of tillers | **11 MAP**  | 0.15*             | 0.09       | 0.05     | -0.14*   | 0.53*     | 1.00     |
| **14 MAP**     | 0.09         | 0.04              | 0.07       | -0.04    | 0.40*    | 0.45*     | 1.00     |
| **17 MAP**     | 0.03         | -0.04             | 0.10       | 0.06     | 0.35*    | 0.45*     | 0.45*    | 1.00     |
| **8 MAP**      | 0.47*        | 0.19*             | 0.04       | -0.11*   | 0.69*    | 0.49*     | 0.30*    | 0.34*    | 1.00     |
| Dry weight     | **11 MAP**   | 0.36*             | 0.29*      | 0.07     | -0.10*   | 0.65*     | 0.59*    | 0.40*    | 0.47     | 0.67*    | 1.00     |
| **14 MAP**     | 0.12         | 0.06              | 0.12       | 0.01     | 0.36*    | 0.45*     | 0.44*    | 0.59     | 0.40*    | 0.59*    | 1.00     |
| **17 MAP**     | 0.00         | 0.01              | 0.11*      | 0.11     | 0.29*    | 0.46*     | 0.48*    | 0.75*    | 0.33*    | 0.49*    | 0.64*    | 1.00     |

All the clones were arranged in descending order and the top 20-25 clones in each trait were shorted out (Table 4) and then searched for common clones that are figured for all the four traits, then for three traits and then for at least two traits and accordingly the best top 15 clones were selected, however, the main weightage was given
Table 4. Selection of top performing palmarosa clones based on their two years field evaluation

| PH | NT | DW | OY |
|----|----|----|----|
| V1P11, V1P26, V1P30, V1P42, V1P69, V5P1, V6P123, V7P1, V7P48, V7P59 | V1P129, V1P130, V2P19, V2P25, V3P24, V3P38, V3P17 | V1P30, V1P131, V2P2, V2P28, V3P21, V3P43, V3P33, V4P47, V4P21, V4P22 | V1P94, V1P28, V1P131, V2P5, V2P33, V3P21, V4P77, V6P39 |

Selection criteria: (PH) Plant height: > 195 cm, (NT) Number of tillers > 270, (DW) Dry weight > 1 kg/plant, (OY) Oil yield > 8 ml/plant

to dry herbage yield. Out of seven germplasm tested, the germplasm population V3 (Variety PRC-1) has thrown 9 out of 15 best clones, followed by two each by V1 and V4 and one each by V6 and V7. However, the germplasm V2 (Udaipur collection) and V5 (Gujarat collection) did not produce any clones in the top 15 clones (Table 5). The V3 is a released variety and is already a superior clone and that might be the reason it has produced a large number of superior segregants through open populations as the female clone always have some added advantages in any breeding scheme because of its well settled combination of genes in the processing of varietal development. So, there is ample scope to further exploit these best clones for the improvement of palmarosa.

Table 5. Average growth and yield characters of 15 best clones of palmarosa

| Best Selection | Clone number | Average plant height (cm) | Average number of tillers | Average fresh herbage yield (kg/plant) | Average dry herbage yield (kg/plant) |
|----------------|--------------|---------------------------|---------------------------|----------------------------------------|-------------------------------------|
| DCM 1          | V1P13        | 204.00                    | 202.50                    | 1.12                                   | 0.81                                |
| DCM 2          | V1P73        | 174.00                    | 260.25                    | 1.86                                   | 0.99                                |
| DCM 3          | V3P3         | 137.75                    | 303.75                    | 1.22                                   | 0.76                                |
| DCM 4          | V3P4         | 157.25                    | 296.50                    | 0.83                                   | 0.50                                |
| DCM 5          | V3P16        | 166.50                    | 253.50                    | 1.04                                   | 0.67                                |
| DCM 6          | V3P20        | 159.00                    | 295.25                    | 1.19                                   | 0.72                                |
| DCM 7          | V3P21        | 149.00                    | 320.50                    | 1.50                                   | 0.93                                |
| DCM 8          | V3P34        | 137.50                    | 152.50                    | 1.54                                   | 0.96                                |
| DCM 9          | V3P39        | 145.25                    | 267.50                    | 1.29                                   | 0.82                                |
| DCM 10         | V3P43        | 158.00                    | 262.00                    | 1.51                                   | 0.93                                |
| DCM 11         | V4P47        | 155.50                    | 227.25                    | 1.37                                   | 0.89                                |
| DCM 12         | V4P84        | 183.00                    | 253.00                    | 1.17                                   | 0.71                                |
| DCM 13         | V3P33        | 143.50                    | 271.25                    | 1.65                                   | 1.01                                |
| DCM 14         | V6P101       | 156.75                    | 173.00                    | 1.12                                   | 0.71                                |
| DCM 15         | V7P58        | 147.25                    | 263.00                    | 1.18                                   | 0.68                                |

Minimum value for each trait: 113.80

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Based on the performance of individual clones in all the seven populations for various traits including herbage and essential oil yield, the best performing top fifteen clones were selected for polycross nursery. The selected clones are labelled as DCM 1 to DCM 15. These clones were intermated in the polycross nursery under natural conditions and putative hybrid seeds were obtained for further evaluation.

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