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Variations in essential oil yield, geraniol and geranyl acetate contents in palmarosa (*Cymbopogon martinii*, Roxb. Wats. var. motia) influenced by inflorescence development

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ABSTRACT

Palmarosa (*Cymbopogon martini* var. motia), an essential oil bearing grass of Indian origin, is highly valued by the cosmetics and perfumery industries for its sweet-smelling essential oil obtained from its inflorescences and leaves. Essential oil from the leaves and inflorescence of fifteen promising palmarosa breeding clones (DCM_1–DCM_{15}) developed, mixture of fifteen clone (DCM_{16}) and a check, variety Trishna (DCM_{17}) was extracted at four developmental stages ((I) unopened spikelets, (II) fully opened spikelets with yellow anthers fully visible, (III) partially mature spikelets with brown and yellow anthers, and (IV) fully mature spikelets showing fully brown inflorescence) in two growing seasons (April–May and September–October). Oil was extracted using hydro-distillation, followed by analysis using gas chromatography/mass spectrometry (GC/MS). Though oil yield in different treatments ranged between 0.85–2.72% on dry weight basis, geraniol (G) content was found to be higher in inflorescence (64.76–76.28% and 63.88–90.59%) and leaves (61.25–92.54 and 76.77–94.03%) of partially and fully mature spikelets, respectively. Biosynthesis and accumulation of essential oil and geraniol were found to increase with maturity, while the trend was reverse in case of geranyl acetate (GA), irrespective of the plant part studied. Though, the amount of oil in inflorescence was higher, when compare with leaves, the geraniol content was higher in case of leaves. Fully matured spikelets with brown inflorescence were found to be the best stage for harvesting to obtain maximum essential oil with geraniol. Some of the promising clones (DCM_2, DCM_4, DCM_9, DCM_{13} and DCM_{14}) were found to have significantly higher amounts of essential oil, geraniol and lower amounts of geranyl acetate than the check variety, Trishna, and thus will have immense commercial values after their release in future for obtaining palmarosa oil.

1. Introduction

Cymbopogon martinii (Roxb.) Wats. var. motia Burk., commonly known as palmarosa or Rosa grass, is a perennial aromatic grass, known for its rose aroma like essential oil. The essential oil obtained from genuine *C. martinii* var. motia is commonly known palmarosa oil in trade, which contains geraniol and geranyl acetate as major compounds. The oil obtained from its leaves and inflorescence (flowering tops) is widely used in soaps, cosmetics, toiletry and tobacco products (Dubey et al., 2000). Palmarosa oil is reported to possess potent antiseptic, mosquito repellent and pain relieving properties and thus has more pharmaceutical significance (Rao et al., 1996). Globally, India has been the major producer and exporter of palmarosa oil (Agarwal, 2008) and Andhra Pradesh, Tamil Nadu, Maharashtra, Uttar Pradesh and Gujarat states are major producers and suppliers of its essential oil from cultivation (Maheshwari and Sethi, 1987; Anon, 2001; Srivastava et al., 2009).

C. martinii var. motia is considered to be superior due to the presence of higher amount of geraniol (85–92%) than *C. martinii* var. Sofia (ginger grass), which contain about 60–70% geraniol. Whole plant of palmarosa contains essential oil, but maximum amount of oil and geraniol are reported in flowering tops (Agarwal, 2008). The characteristic odour of palmarosa oil is attributed to the high content of geraniol and varying amounts of geranyl acetate (Husain, 1994). The inter-conversion of geraniol and geranyl acetate was found to be dependent on a complex interplay of different regulatory controls operating at the organ, cellular and enzyme levels and the ratios of geraniol and geranyl acetate fluctuated during plant development (Dubey and Luthra, 2001). The amount of geranyl acetate decreased from 59.0% to 3.0%, whereas that of geraniol

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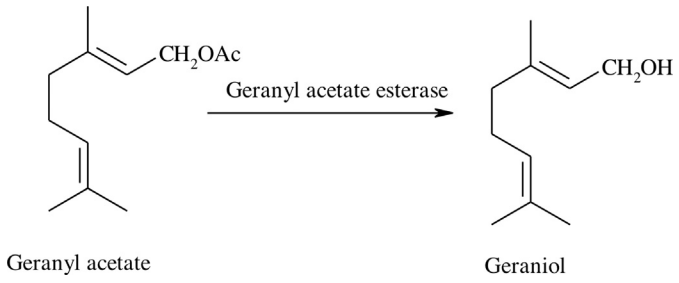

Fig. 1. The biotransformation of geranyl acetate into geraniol (Dubey and Luthra, 2001).

increased from 33.0% to 91.0% indicating the role of an esterase involved in the conversion of geranyl acetate to geraniol during leaf development (Dubey and Luthra, 2001; Ganjewala and Luthra, 2009). Dubey and Luthra (2001) reported that in developing palmarosa inflorescence, a substantial amount of geraniol underwent acetylation to form geranyl acetate, which in turn got hydrolyzed by geranyl acetate esterase to produce geraniol as the inflorescence matured (Fig. 1). Geraniol and geranyl acetate are two major compounds in the palmarosa oil and has commercial value in cosmetic and perfumery industries (Agarwal, 2008).

Harvesting of palmarosa at flowering stage to obtain maximum essential oil is a known (Sharma et al., 2009), however the accurate stage during inflorescence development to obtain maximum essential oil and geraniol from both, leaves and inflorescence is not clear. Moreover, influence of seasons on the essential oil yield, geraniol and geranyl acetate in both leaves and inflorescence has to be understood to obtain quality products to the user industry.

Palmarosa is one of the essential oil bearing industrial crop and its breeding study is also required to obtain the improved clones having better amount of the essential oil, geraniol and geranyl acetate than check which can be used for cosmetic and perfumery purpose and farmer's higher income. This study will be helpful to determine the optimum stage for harvesting of palmarosa for obtaining maximum amount of essential oil, geraniol and geranyl acetate for better crop's productivity and profitability and to identify superior clones for further crop improvement. Thus, it was thought to understand the effect of aging of inflorescence and leaves of palmarosa on the essential oil yield and its two main compounds, geraniol and geranyl acetate by obtaining essential oil from inflorescence and leaves using hydro-distillation method at different growth stages and quantifying compounds by GC/MS.

2. Materials and methods

2.1. Experimental site

The experiment was conducted at the Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat, India for two harvesting seasons during the year 2013. The experimental farm is located at 22°35′N and 72°55′E, at an altitude of about 45.1 m above mean sea level.

2.2. Plant material

The composite hybrid seeds of palmarosa were collected separately from each clone of polycross nursery raised in the field at Directorate. Experimental material included fifteen promising clones (DCM_1– DCM_{15}), a mixture of all fifteen clones in equal proportions (DCM_{16}) and check variety, Trishna (DCM_{17}). Fifteen best palmarosa clones were identified by the two years screening of 1200 plants of 7 germplasm for different growth, yield and quality parameters. These fifteen clones were planted in the polycross nursery in ten replications, randomized in such a way that each clone will get an equal chance of out crossing. The composite hybrid seeds of palmarosa were collected separately from each clone from the polycross nursery. The experiment was laid out in randomized block design (RBD) with three replications. Freshly collected seeds were sown in the nursery beds in June, 2012 during the onset of monsoon. One month old healthy seedlings were transplanted to the main field in plots of 6 m × 5 m size at a spacing of 1 m × 1 m. The crop was raised following the standard agronomic practices. No morphological differences among the clones were observed. First and second harvesting were done during April–May and September–October, 2013, respectively.

The fresh inflorescence and leaf biomass of the tagged plants were harvested at defined interval during both the seasons, representing four developmental stages (Fig. 2) namely stage I (unopened spikelets), stage II (fully opened spikelets with yellow anthers fully visible), stage III (partially mature spikelets with brown and yellow anthers in colour) and stage IV (fully mature spikelets showing brown inflorescence) for extraction of essential oil from different clones and quantification of geraniol and geranyl acetate in the oils.

2.3. Extraction of essential oil

Freshly harvested leaves and inflorescence (200 g each) were hydro-distilled for 3 h in a Clevenger-type apparatus in triplicate. The distillate was extracted with diethyl ether and the etheral layer was dried over anhydrous sodium sulphate. Ether was distilled off on gently heated water bath and oils were stored in amber vials at 4–8 °C until analysis.

2.4. Gas chromatography–mass spectrometry analysis of essential oil

Analysis of the volatile oils was performed on a GC/MS (Focus–Polari Q) bench top ion trap mass spectrometer equipped with a ZB-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Chromatographic conditions were as follows: injector temperature- 200 °C, carrier gas- helium, flow rate- 1 ml/min, injection volume- 1 μl (1 μl oil in 1 ml). The column temperature was held at 45 °C for 5 min, and programmed at 4 °C per minute to 200 °C and held for 1 min with split flow (1:70). The temperatures at MS transfer line and source were 250 °C and 200 °C, respectively. The GC column was coupled directly to the spectrometer in EI mode at 70 eV with the mass range of 40–500 a.m.u at 1 scan/s. Individual compounds were identified by mass spectra and their identities were confirmed by comparing their mass spectra with Mass Spectral Library (Ver. 2, 2005) and literature (Adams, 2007).

2.5. Statistical analysis

The analysis of variance was done in randomized block design for various observations recorded during experiment by using statistical software SAS 9.2 (Anon, 2008). Comparisons among the essential oil, geraniol and geranyl acetate obtained from the clones including check and between the harvesting stages. The results were presented at 5% level of significance ($P = 0.05$). The critical difference (CD) values were calculated to compare the various treatment means.