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Crop adaptation and modeling for prediction of essential oil production and quality of a geraniol rich strain of *Cymbopogon commutatus* (Steud.) Stapf [RL(J) CC1] using energy indices

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ABSTRACT

Occurrence of gerniol and geranyl acetate as major chemical constituents were reported in the essential oil of *Cymbopogon commutauts* (Steud.) Stapf. A selectant having H"80% total geraniol and coded as RRL (J) CC1 has been studied by way of quantifying growth response coefficient values of morpho-economic character, which was 1.0 signifying its good adaptability under subtropical environment. Leaf adaptation has been quantified as phyllochron which exhibited 519.6 and 775.6 degree-days for production of single mature leaf during first and second harvests, respectively. Floral adaptation was quantified as photoperiod response coefficient b=412 (degree-days/day-length). Based on two years pooled observation (2001-2002), regional crop models have been developed for prediction of essential oil production and its quality as total geraniol content (%) by using most-efficient energy indices viz., phenothermal index and thermo/photo ratio.

Key words: Adaptation, *Cymbopogon commutatus*, Degree-days, Energy summation indices, Geranyl acetate, Heat use efficiency, Phenothermal index, Phyllochron, Thermo/photo ratio.

Cymbopogon commutatus (Steud.) Stapf. yields essential oil having geraniol and geranyl acetate (62.40%) alongwith citral (18.33%). It differs chemically from Sudanese *C. commutauts* which contains unusual menthadienols (89%) (Benthorpe, *et al.,* 1976, Corrigan, 1992). Its phenological development is regulated by plant age and environmental condition as of which temperature is the most important (Bauer *et al.,* 1984). Day-length is a primary factor in inducing plants to develop reproductive structures on the apex. The impact of these factors can alter and interact with the plant to establish jointly a developmental pattern at geographical location site. Phenology provides a major control over adaptation and yield (Coa and Moss, 1989). Leaves are the main source of essential oil for *C. commutatus*. Therefore, its adaptability has to be quantified by way of studying phyllochron (plant development as degree-days needed to produce a mature leaf) in relation to ambient temperature which aid in making crop management decisions.

Keeping in view the above facts, the study was undertaken to quantify the effect of ambient temperature and day-length on essential oil content and its quality as total geraniol content (geraniol + geranyl acetate; %).

MATERIAL AND METHODS

Plant material and study site

A U.S. patented drought resistant selectant RL(J)CC1, isolated from *C. commutatus* having better quality of essential oil in terms of total geraniol content (≈80%)and low citral content (3-5%) was taken up for study at Field Research Station of IIIM, Jammu [32°44' latitude (N) and 75°55' longitude (E) at altitude ≈300m above sea level] on soil with pH 7.92 sandy loam texture, organic carbon (0.25%), available nitrogen (118 kg ha^{-1}), available phosphorus (20.01 kg ha^{-1}) and available potash (160.20 kg ha^{-1}). The experiment was laid out in randomized block design replicated thrice each measuring 666.66 sq. m having 4167 plants at spacing of 40×40 cm row to row and plant to plant. The crop was planted on 15 January 2001 vegetatively through slips Two dates of harvest viz; 15 June and 15 September (2001-2002) for the two consequentive years were taken to cover vegetative to blooming phenophases during the growing period. Ten plants per replication were harvested at each phenophase for recording the morpho-economic parameters. The recorded data were subjected for pooled analysis as suggested for perennial crops (Despekhov, 1984) and then used for mathematical modeling (Linear and multiple regression equations).

Isolation of essential oil

Samples of fresh herbage in triplicate each weighing 500g were used for determination of essential oil content by hydrodistillation method using Clevenger-type apparatus (Clevenger, 1928). Sub-samples were used for dry matter content.

Table 1: Adaptive values as growth response coefficient of *C. commuatatus* [RL(J)CC1]

Growth Indices	Growth Response Coefficient (b)
Height Growth Index	1.0
Tiller Growth Index	1.0
Herbage Growth Index	1.0
Essential Oil Growth Index	1.0

Analysis of essential oils

The essential oil was subjected to qualitative and quantitative analysis by [FC and FC-MS, Hewlett-Packward MSD 5971 A, 60M×0.25mm, 0.32um DB wax fused silica capillary column. It was programmed for 60°-240≈C at 4°C/ min., injection temperature 240°C by the split sampling technique. Split ratio 2:1000, carrier gas helium 2ml/min. transfer line temperature 250°C, ion source temperature 170°C, ionization energy 70 eV]. The oil constituents were identified by comparing their retention indices and mass spectra with library spectra at Dragoco Holtzminden.

Phenology

Phenological time scale for describing the phenophases numerically coded with the appearance of vegetative and reproductive phases (Shahi *et al.,* 2005) and its further division into demical fraction was as follows: 2.0=two leaves/ tiller; 3.0=three leaves/tiller; 3.5=three mature leaf and fourth one at development; 4.0= four leaves/tiller; 4.5=four mature leaf and fifth at development and elongation along with flower initiation. 5.0 = five leaves/tiller; 5.5 = five leaves mature and sixth at development and elongation, 6.0 = six leaves/tiller and appearance of culm and >57% flowers, 6.5 = six mature leaves and seventh leaf development and elongation, floral induction and mature leaf senescence.

Determination of adaptive values

The morpho-economic characters were quantified as growth response coefficient values (b) for plant height (cm), tiller/plant (nos), herbage/plant (g) and essential oil production/plant (g) by regressing their relative index values as obtained from the mean of all entries at harvest date minus grand mean and termed as Height Growth Index, Tiller Growth Index, Herbage Growth Index and Essential Oil Growth Index, respectively (Table 1). b=1 means plant responds similarity with respect to different phenophases/growth stages (Pederson *et al.,* 1991).

Energy summation indices

Degree-days (°Cdays) was computed by the following Shahi and Singh, (1987) Phenothermal index photothermal unit, heliothermal unit and heat use efficiency were computed following methodology given by (Chakravarty et al., 1984). The thermo/photo ratio was calculated as the degree-days per day divided by the day-length in hours (Cao and Moss, 1989). The impacts on essential oil production and its quality were determined and expressed by their respective regression coefficients.

Phyllochron

The thermal requirement for production of mature leaf during the successive phenophases at 1st and 2nd harvest have been quantified and termed as Phyllothermal response coefficient. Validation of the models were done by calculating the correlation coefficient (r) between predicted and observed values and their significance at 5% probability level.

Model development for prediction of essential oil quality as total geraniol content (%)

Pooled analysis of two years data (2001-2002) for essential oil production (g plant^{-1}) and its quality as total geraniol content (%) were done (Despekhov, 1984). These dependent variables along with most efficient energy indices having low coefficient of variation (%) viz., thermo/photo ratio and phenothermal index (Table 3) as independent variables have been used for both harvests separately in linear regression equations and validated by correlating the predicted and observed values. The combined effects of phenothermal index and thermo/photo ratio were subjected to multiple regression with essential oil production to predict oil quality as total geraniol content (%) and their strength evaluated by index of agreement (d) (Willmott, 1981).

$$d = 1 - \left[\frac{\sum_{i=1}^{n} (P_i - O_i)^2}{\sum_{i=1}^{n} P_i' + O_i')^2} \right]$$

Where

O = Observed variables, P = Predicted Variables, n = number of observations, P_i' = P_i-O, and O_i' = O_i – O, slashes (\) indicates absolute values. A 'd' value of 1 indicates complete agreement between predicted and observed values.

RESULTS AND DISCUSSION

Adaptation

Growth response coefficient values (b=1) have been quantified for each morpho-economic characters viz height

Table 2: Linear descriptive statistics between leafing and degree-days for *C. commutatus* [RL(J)CC1]

Harvest(s)	Phyllothermal response coefficient (b)	Regression constant (a)	Correlation coefficient (r)*	Coefficient of determination ($r^2 \times 100$)
Ist	519.60	-436.40	0.995	99.00
IInd	775.60	142.31	0.999	99.80

Table 3: Coefficient of variation (CV%) values of energy summation indices at each harvest of *C. commutatus* [RL(J)CC1]

Energy Indices	Coefficient of variation (CV%)	
	Harvest	
	Ist	IInd
Heliothermal units	38.96	40.96
Photothermal units	29.87	29.63
Degree-days	29.77	27.08
Heat use efficiency	20.00	26.92
Phenothermal index	17.03	12.64
Thermo/photo ratio	16.00	16.40

growth index, tiller growth index, herbage growth index and essential oil growth index.

Leaf adaptation has been quantified as phyllocron (degree-days/leaf) which were 519.6 and 775.6 degree-days for production of single mature leaf during first and second harvests, respectively. The phyllothermal response coefficient (b) given in Table 2 measures the response of leafing to ambient temperature and was found to be more sensitive during second harvest due to comparatively higher b value (b=775.6). It showed that phyllochron response altered the essential oil production as obtained from the leaves during crop development.

Floral adaptation was represented by occurrence of two dates of flowering. Appearance of first period of flower initiation was observed at 4.5 leafing stage having day-length period of 12 hours and its induction continued upto 6.5 leafing stage (>75% blooming). The mean fresh herbage ranging between 475-500 and 600-625 (g plant^{-1}) was obtained at first and second harvest respectively. The floral adaptation and its extent of photoperiodic response was quantified and termed as photoperiodic response coefficient. After taking the first harvest at 4.5 leafing stage, the flower initiation was observed during first week of July at 5.5 leafing having day-length 13.4 hours and continued upto mid September (Post-inductive phase).

Energy indices during growth period

Out of six energy indices viz., heliothermal units, photothermal units, degree-days heat use efficiency, phenothermal index and thermo/photo ratio, the most efficient were the phenothermal index and thermo/photo ratio having comparatively low Coefficient of Variation (CV%) values 17.03% and 12.64% followed by thermo/photo ratio having 16.00 and 16.40% during first and second harvest, respectively (Table 3).

Phenothermal index and thermo/photo ratio vs essential oil production and quality

Based on pooled analysis of two year's data, a linear response to phenothermal index and thermo/photo ratio on essential oil production and its quality as total geraniol content were related to phasic development, suggesting that the heat unit concept is appropriate. Therefore linear regression equations were developed for prediction of essential oil production (g plant^{-1}) and its quality as total geraniol content (%) as dependent variables against phenothermal index as independent variable by way of quantification of their b values and termed as Phenothermal essential oil growth response coefficient. Its responsiveness for production of essential oil growth was 0.373 and 1.011 per unit phenothermal index at first and second harvests respectively (Table 4). The impact of essential oil growth response coefficient was higher for the production of total geraniol content at first harvest which signified its positive responsiveness during the harvest scheduling (Table 5).

Models for prediction of essential oil production and total geraniol content (%) as dependent variables by using most efficient energy indices i.e. thermo/photo ratio and phenothermal index jointly alongwith essential oil production (as independent variables) in multiple regression equation at first and second harvest were developed.

The multiple regression equations are as under:

$Y_{TG\,(I)}$ = -24.950 - 6.276 × Essential oil production (g/plant) + 68.915 × Thermo/photo ratio + 3.838 × Phenothermal Index

$Y_{TG\,(II)}$ = -85.38 – 9.874 × Essential oil production (g/plant) + 100.415 × Thermo/photo ratio + 4.571 × Phenothermal Index

Table 4: Linear description between phenothermal index and its impact on essential oil production and total geraniol content (%) at 1st and 2nd harvests of *C. commutatus* [RL(J)CC1]

Dependent variables		Phenothermal essential oil growth response coefficient (b)	Regression constant (a)	Correlation coefficient (r*) values for model validation
Essential oil	Y_{EOI}	0.373	-1.886	0.997
production (g/plant)	Y_{EOII}	1.011	-12.745	0.799
Total geraniol	Y_{TGI}	5.627	20.380	0.962
content (%)	Y_{TGII}	3.000	20.400	0.950

Table 5: Linear description between thermo/photo ratio on essential oil production and total geraniol content (%) at 1st and 2nd harvests of *C. commutatus* [RL(J)CC1]

Dependent variables		Thermo /photo ratio essential oil growth response coefficient (b)	Regression constant (a)	Correlation coefficient (r*) values for model validation	Index of agreement
Essential oil	Y_{EOI}	5.991	-2.912	0.965	--
production (g plant^{-1})	Y_{EOII}	12.133	-10.883	0.947	--
Total geraniol	Y_{TGI}	93.683	2.364	0.974	0.994
content (%)	Y_{TGII}	15.668	54.127	0.770	0.949

Y_{EOI} & Y_{EOII} = Essential oil production at 1st and 2nd harvest, respectively.
Y_{TGI} & Y_{TGII} = Total geraniol content at 1st and 2nd harvest, respectively.
* Significant at 5% probability level.

Table 6: Essential oil constituents of *C. commutatus* [RL(J) CC1]

RI	Constituents	Percentage
564	Geraniol	64.26
808	Geranyl acetate	15.79
972	2-Caren-4-ol	0.08
1112	Geraniol	2.65
1304	Geranyl Formate	0.07
1899	β-elemen	0.13
1900	Caryophyllene	0.81
1904	Cadinen delta	0.08
2065	Candinen butyrate	0.37
2252	Elemol	0.91
2344	Elemicin	0.03
2366	α-Eudesmol	0.06
2540	Carpronsaure geranyl ester	0.50
3746	Caren (2)	0.41
5549	Geraniol 6,7-epoxid	0.07

Where,

$Y_{TG (I)}$ and $Y_{TG (II)}$ are total geraniol content at first and second harvest, respectively.

Validation of these models was done using correlation coefficient and Index of agreement (Table 4). Developed regional models are useful for determination of essential oil production as well as its quality (as total geraniol content (%)) at various phenophases/growth stages by using efficient energy indices viz., phenothermal index and thermo/photo ratio either singly or in combination. Phenology model has identified two distinct growth stages i.e. 4.5 and 5.5 leafing stages for getting optimal oil yield in accordance with better quality as total geraniol content (%) at first and second harvests, respectively. First and second harvests yield 125 kg oil ha^{-1} and 150 kg ha^{-1} at second along with 84% and 77.68% of total geraniol content, respectively. The average essential oil recovery 0.4% (w/w; fresh weight basis) was obtained during both the harvests. However 20% increase in essential oil and fresh herbage at second harvest was recorded. This may be attributed to production of more tillers on the mature clumps without any tiller mortality. Oil quality as total geraniol content (%) was comparatively as low as 6.33% at second harvest possibly due to diffused sun light and comparatively lower bright sunshine hours during the monsoon season. Similar results have been reported on a citral rich cultivar of *Cymbopogon pendulus* (Nees ex. Steud.) Wats i.e. "Jammu Lemongrass" under subtropical environment (Shahi *et al.,* 1981). The essential oil composition of a representative sample of both harvests exhibited better quality due to the presence of 80% geraniol content (Geraniol 64.26% and Geranyl acetate 15.79%) (Table 6).

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