

**CHARACTER ANALYSIS ON
NATURAL AND X-IRRADIATED POPULATION OF
JAVA CITRONELLA (*Cymbopogon winterianus* Jowitt)**

**A Thesis
Submitted to the
Bidhan Chandra Krishi Viswavidyalaya
for the award of the Degree of Doctor of Philosophy
in
GENETICS AND PLANT BREEDING**

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March, 1983

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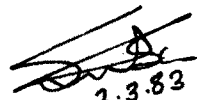
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CERTIFICATE

This is to certify that the work recorded in the thesis entitled CHARACTER ANALYSIS ON NATURAL AND X-IRRADIATED POPULATION OF JAVA CITRONELLA (Cymbopogon winterianus Jovitt) submitted by Shri Chittaranjan Kole for the award of the Degree of Doctor of Philosophy in Genetics and Plant Breeding of the Bidhan Chandra Krishi Viswavidyalaya, is the faithful and bonafide record of research work carried out under my personal supervision and guidance. The results of the investigation reported in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.


2.3.83
(Subir Sen)

ACKNOWLEDGEMENT

I feel elysian pleasure to consecrate my fathomless regards and pronounced gratitude to Dr. Subir Sen, the Professor of Genetics and Plant Breeding and Dean, Post-Graduate Studies of our Viswavidyalaya, for his invaluable guidance and auspicious supervision ab initio till completion of this research pursuit.

I am immensely indebted to Dr. S.C.Rakshit, Reader and Head and Dr. S. Dana, Reader and formerly Head of the Department of Genetics and Plant Breeding for sustaining keen interest and for their ultra valorem technical advices whenever desiderated for.

I would like to convey my deepest sense of respect and infinite gratitude to Dr. Akhtar Hussain, Director, and Mr. O.P.Virmani, Head of Information, Liaison and Extension Discipline of Central Institute of Medicinal and Aromatic Plants (C.S.I.R.), Lucknow and Dr. M.R.Narayana, Scientist-in-charge of the Bangalore Regional Centre of the same organisation for extending library facilities, supplying some of the literature and providing the propagules for the first experiment of this endeavour.

I do intend to record the kind co-operation of Dr. S. M. Chatterjee, Director and Dr. S. L. Basak, Head of the Botany Division of Indian Jute Research Institute, Barrackpore, where the 'bibits' were X-irradiated.

I am appetent to express my gratefulness to Dr.S.P.Popli, Scientist-in-charge, Medicinal Chemistry Division, Central Drug Research Institute, Lucknow for lending commiserative help in GLC analysis of oil in the Laboratory of the Regional Sophisticated Instrument Centre (C.S.I.R) at that Institute.

My sincere regards and gratitude are for Prof.S.C.Bhattacharya,

Director, Bose Institute for the favour of his allowing consultation of some periodicals in the Library and for Dr. A. Chatterjee, Scientist-in-charge, Regional Sophisticated Instrument Centre (C.S.I.R.) Calcutta, for his help with respect to GLC analysis of oil.

Infinitum regards and heartiest gratefulness are due to Mr. Anand Swarup, IAS, Vice-Chancellor, Dr. V.K. Srivastava, Mr. P.G. Biswas and Mr. S. Biswas of the Computer Section of G.B.Pant University of Agriculture and Technology for extending the computer services to process the data of the first experiment of this work.

Byoyant-heartedly I convey my candid gratitude and regards to Prof. S.B.Chattopadhyaya, Chief Advisor, Department of Agriculture, Government of West Bengal, Prof. M.M.Chakravorty, Vice-Chancellor, Jadavpur University and Prof. H. Dutt, Emeritus Scientist, I.C.A.R., Prof. L.N.Mandal, Prof. N.A.Choudhuri, Prof. S. Mukhopadhyaya of this University for their affectionate inspiration and counsel which had been of extreme help throughout the investigation.

The cordial inspiration and warm affection of Dr. Roma Choudhuri, Ex-Vice-Chancellor, Rabindra Bharati, Dr. Manmatha Roy, the celebrated Drama Writer, and Mr. Joydev Mukhopadhyay of this University are deeply acknowledged.

Sincere thanks are also due to the teachers, staff-members, students, my colleagues and confreres as well as my well-wishers, known or unknown, who had helped me directly or indirectly during the studies.

In fine, special acknowledgement is, hereby, sincerely made to the C.S.I.R. for awarding me the Junior and Senior Research Fellowship for conducting this research work.

Dated : Kalyani,
March , 1983

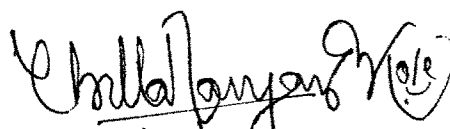

(CHITTARANJAN KOLE)

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ABBREVIATIONS USED

BCKV	Bidhan Chandra Krishi Viswavidyalaya
CINAP	Central Institute of Medicinal and Aromatic Plants
CIMPO	Central Indian Medicinal Plants Organisation
CSIR	Council of Scientific and Industrial Research
DAT	Days after transplanting
DES	Diethyl sulphate
DMS	Dimethyl sulphate
DMSO	Dimethyl sulphoxide
EMS	Ethyl methane sulphonate
GLC	Gas-liquid chromatography
ISI	Indian Standards Institute
LD ₅₀	Fifty per cent lethal dose
LD ₁₀₀	Hundred per cent lethal dose
RRL	Regional Research Laboratory
VM ₁	First vegetative irradiated generation
VM ₂	Second vegetative irradiated generation

FOREWORD

Citronella oil, the essential oil of paramount commercial importance, is obtained from two taxonomically and chemotaxonomically proximal but morphologically and physiologically distinguishable species. One is Cymbopogon nardus Rendle, lenabatu (Andropogon nardus ceylon de Jong), the so-called "lenabatu", "new grass" or "Ceylon citronella", which contributes the bulk of "Ceylon oil" and proportionately a very small quantity of "Java oil". The other is Cymbopogon winterianus Jowitt, mahapengiri (Andropogon nardus java de Jong), the so-called "mahapengiri", "Old grass", "Winter's grass" or "Java citronella", which produces the bulk of "Java oil" and proportionately a very small amount of "Ceylon oil" (Guenther, 1950). Java citronella is superior to Ceylon citronella in terms of oil yield as well as oil quality, particularly for higher content of the commercially important principles, e.g., geraniol, citronellal and citronellol (Guenther, 1950; Anon, 1950; Bor, 1960; Virmani et al., 1967b; Virmani and Datta, 1971; Virmani et al., 1979 and other workers).

Both the species of citronella belong to genus Cymbopogon Spreng. of tribe Andropogoneae under group Panicoideae of family Gramineae (Bor, 1960). According to Guenther (1950) all the cultivated types of citronella are descendants of C. confertiflorus Stapf, the so-called "mana" grass, which occurs wild in Ceylon. Bor (1960) also considered C. nardus var. confertiflorus (Steud.) Stapf to be the parent of both C. nardus and C. winterianus.

"Mahapengiri" was introduced from Ceylon, its centre of origin, in Java (1889), Taiwan (1912), Central American States of Honduras and Guatemala (1940) and later on in other South American countries.

Present world production of citronella oil amounts to about 5,000 tonnes mainly contributed by Taiwan, Guatemala, Honduras, Malaya, Brasil, Ceylon, India, Argentina, Equador, Madagascar, Mexico and West Indies. Introduction of citronella in India dates back to 1959 when a few slips had been collected from Java by National Botanical Research Institute. Albeit successful acclimatization at Lucknow, the introduction was not followed up and was totally lost due to flood. Fortunately, a simultaneous introduction of a few slips from Java by Cinchona Department, Ootacamund, through the courtsey of Essential Oils Research Committee, CSIR, helped the establishment and extension of this crop in India by CIMPO (presently CIMAP) Regional Centre at Bangalore in 1961. In India, 300-350 tonnes of oil had been produced for the last 6-8 years prior to 1979 in the States of Assam, Karnataka, Maharashtra, Tamil Nadu, Uttar Pradesh and West Bengal (Virmani et al., 1979).

Statistics of import-export of different essential oils for 1960-'61 to 1976-'77 (Anon, 1968-'77, Virmani and Datta, 1971) reveal that India had been successful to dispense with import of citronella oil only since 1974-'75. Narayana et al. (1976) observed an apparent surplus availability of this oil in Indian market since 1974-'75 in the perspective of the size and needs of the essential oil for the existing industries. They also extended several suggestions so as to boost up the national export potential in respect of citronella oil.

Java citronella oil contains an array of chemical constituents (Guenther, 1950, 1968; Anon, 1950; Virmani and Datta, 1971; Virmani et al., 1979) of which commercially most important principles comprise of citronellal, geraniol and citronellol. The oil serves as a starting

material for extraction of various important isolates, such as, geraniol and citronellal, which can be further converted into some of the extensively used aromatics, such as, citronellol, hydroxycitronellal, synthetic menthol, esters of geraniol and citronellol, etc. This oil finds its use also in scenting of soaps, mosquito repellent creams and other technical preparations (Guenther, 1950; Anon, 1950; Bor, 1960; Virmani et al., 1979). This oil also possesses appreciable medicinal potentialities (Suri et al., 1978).

With the ever increasing demand of this oil for diverse industrial products (Virmani et al., 1979), it is imperative that the projected surplus shown in 1974-'75 has dwindled down to the level where the total oil produced in India might just meet the need for the industries. The possibility of extension of area of cultivation to balance the need appears to have reached its limit vis-a-vis the competition on lands occupied by food grains and plantation crops. The improvement of essential oil yield per unit area as well as tolerance to minimum cultivation would go a long way to establish this crop as an important cash crop in the limited area.

In spite of its immense economic importance not much work has been done so far for improvement of this crop through applied genetics (Chauhan et al., 1976; Gupta & Jain, 1978; Choudhary and Kaul, 1980a). Whatever information is available is not inadequate to indicate the possibility of genetic upgrading, particularly in the vegetatively propagated Java citronella. Attempts have been made in this treatise to evaluate the exploitability of natural genetic resource for identification of improved genotypes. The possibility of selection for beneficial genotypes for oil yield has also been studied from mutated clones subjected to dechimerization and selection.

SECTION - I

**CHARACTER ANALYSIS ON
NATURAL POPULATION OF JAVA CITRONELLA
(Cymbopogon winterianus Jovitt)**

INTRODUCTION

Free intra- and interspecific hybridization (Bor, 1953; Gupta, 1971) have reportedly resulted in a swarm of transition forms in general in the genus Cymbopogon Spreng. (Anon, 1950) and in C. winterianus Jowitt also (Ravikumar and Narayana, Personal Communication). Besides, this species had been under natural and concomitant artificial selection while grown in various countries in diverse edapho-climates. Thus, free hybridization coupled with selection pressures had most plausibly yielded minor evolutionary changes, which, in fine, resulted in various genetically stabilized, morphologically discernible and economically distinct types or cultivars in this species (Kole et al., 1981b). To date, in India, there are four such exotic types or cultivars introduced from Java (Virmani et al., 1979), later on discriminated into Java-I or Java (Type-I) and Java-II or Java (Type-II) (Rajamani et al., 1965), from Guatemala (Naryanana and Ganesha Rao, 1969) and from Burma (Kaul et al., 1977), hereinafter denoted as Java-I, Java-II, Guatemala and Burma respectively.

Comparative assessment was reported in respect of general performance, yield and composition of oil between Java-I and Java-II by Virmani et al. (1967b, 1979) and among Java-I, Java-II and Guatemala by Narayana et al. (1976). Kaul et al. (1977) compared Burma with Java-II (cited as 'Burmese' and 'Java' respectively) in terms of apparent growth and vigour, herbage yield per plant, oil percentage and oil constituents. Ravikumar and Narayana (Personal Communication) depicted the distinguishing morphological features of Java-I, Java-II and Guatemala to facilitate identification. They evaluated the quality of oils also. Kole et al. (1981b) studied the morpho-economic features of Burma, wherein differences from other three cultivars were also cited.

Loustalot and Fernandez Pol (1949) reported the differences of 'Java' and Guatemala for yield of fresh grass, yield and percentage of oil and geraniol content in oil at different heights as stage of harvest.

But in all those studies except that of Loustalot and Fernandez Pol (l.c.) intervarietal differences of the characters were not subjected to statistical estimation but were observed at visual and or numerical level.

A statistical approach was undertaken at Bangalore Regional Centre of CIMAP with a view to estimating the nature and extent of the natural intra-varietal variation of eighteen characters and intervarietal variation of twenty characters in four cultivars, viz., Java-I, Java-II, Guatemala and Burma (Kole et al., 1980).

(The objective of the present study is to estimate the genetic base of variation in and among the cultivars, to identify the best assemblage of morpho-economic characters for effective selection response of the genotypes and to apply selection procedure on that basis to isolate the biologically competent genotype for future breeding works.)

Estimates of the genetic parameters, such as, genotypic coefficient of variation, genetic advance and heritability are basic to any selection programme. The present author is not aware of any such information in this crop.

(The present experiment encompasses, in its second step, estimation of these genetic parameters and therefrom, the prediction of the relative worth of some characters in selection.)

Oil yield in citronella, similar to other crops, is a quantitative

character, which, most often, registers low to moderate heritability. This character would most likely succumb to environmental interactions. Hardly reliable and effective would be this character as a selection criterion. The component characters contributing to this complex character may very well be exploited in the alternative approach based on "correlation and causation" proposed by Wright (1921 a). Such a component approach has been attempted with two steps. (The first step aims at determination of the nature and extent of correlations among oil yield from leaf and the probable contributing characters. The second one involves in the estimation of the direct and indirect contributions of these characters toward oil yield from leaf.) These findings have been utilized to identify the characters worthwhile to be effective and profitable basis of selection for genetic upgrading of this crop.

REVIEW OF LITERATURE

According to Virmani et al. (1979) citronella was introduced in India from Java in 1959 at the National Botanical Research Institute. The plants acclimatized successfully in a small scale plantation at Lucknow. But this introduction was not followed up as the crop perished in flood.

Java citronella was simultaneously introduced in India by the Cinchona Department, Ootacamund in around 1959 (Virmani et al., 1979). According to Narayana et al. (1976) the Bangalore Regional Centre of CIMPO (presently CIMAP) had obtained these slips in 1962 in two consignments, which appeared to be a single strain, viz., Java citronella. But while grown at Bangalore, these exhibited a considerable variation and was discriminated into two cultivars, viz., Java-I and Java-II. A subsequent introduction of another sample of Java citronella slips was made from Guatemala in Central America. Repeated observations proved Java-II to be the best amongst three cultivars. The oil of Java-II gained acceptance from perfumery industry and it was, therefore, selected for large scale cultivation.

Virmani et al. (1967b) reported Java-I and Java-II to contain alcohols calculated as geraniol to the extent of 71.24% and 65.18% respectively while aldehydes calculated as citronellal to the extent of 13.85% and 37.0% respectively.

Loustalot and Fernandez Pol (1949) conducted an experiment to study the effect of harvesting citronella ('Java' and Guatemala) at three different heights (minimum - 1.00 m, medium - 1.35 m and maximum 1.83 m) and the three numbers of harvest (8, 11 & 15) over a period of four years on yield and oil content. In all the treatments, Java

surpassed Guatemala in respect of production of fresh grass. Guatemala surpassed Java with respect to percentage of oil, as a result of which Guatemala produced higher average yield of oil per hectare than Java while harvested at maximum or low height. But the result was reverse when harvested at medium height since percentage of oil in Guatemala was not much higher than that in Java when harvested at medium height, and the yield of grass was higher in Java than that of Guatemala. Guatemala was slightly but consistently superior with regard to percentage of geraniol.

Introduction and chemical evaluation of 'Burmese citronella' was reported by Kaul *et al.* (1977). Planting materials were obtained through the courtesy of Dr. E. K. Janaki Ammal from collections maintained at the Botany Department of Madras University. Those were planted in experimental plots of RRL, Jammu, along with 'Java'. The cultivars appeared to be morphologically similar. But 'Burmese' had an edge over 'Java' in growth and vigour. Oil content of 0.8% was same for both the cultivars. However, GLC analysis of oil revealed accountable differences. The percentages of citronellal, citronelloi, geraniol and geranyl acetate were 66.3, 6.4, 14.7, 4.2 respectively for 'Burmese' and 48.6, 14.2, 29.6 and 0.5 respectively for 'Java'. Hence, 'Burmese' was claimed superior to 'Java' as a source for citronellal.

But in all of these studies only the economic characters, *viz.*, fresh herbage yield, oil yield, oil content and oil composition were taken into consideration.

Ravikumar and Narayana (Personal Communication) subsequently carried out a comprehensive comparative study on some economic as well

as morphological characters of Java-I, Java-II and Guatemala along with Ceylon citronella (C. nardus Rendle). They studied on the "nature of the plant, number of vegetative shoots/clump, number of flowering shoots/clump, nature of the leaf blade, colour of the leaf blade, flowering, duration of flowering, nature of flowering shoots, length of the panicle, nature of the panicle, arrangement of the main lateral branches on panicle axis, number of spikelets per raceme, lodicules & style". The qualitative studies of oils revealed the contents (per cent by weight) of total alcohols calculated as geraniol to be 68.0, 94.0 and 90.8 and total aldehyde calculated as citronellal to be 18.1, 44.1 and 37.9 for Java-I, Java-II and Guatemala respectively as against 85-97% total alcohols calculated as geraniol and 35-45% total aldehyde calculated as citronellal stipulated by ISI (Anon, 1957).

All the reports except that of Loustalot and Fernandez Pol (1949) were based on visual and or comparison of the numerical records of observation. The retrospect reveals two facts, i.e., (i) test of statistical significance of the observed intervarietal differences for all important characters is still lacking; (ii) a handful of other characters which may have important impact on economic product awaits statistical evaluation.

The author conducted (Kole et al., 1980) an experiment at Bangalore Centre of CIMAP to study the natural variation in four cultivars, viz., Java-I, Java-II, Guatemala and Burma. Apart from the sixteen morphological characters considered in the present experiment, percentage and yield of citronellal and geraniol were also included in that experiment.

No information, whatsoever, is available regarding the estimates on genetic parameters, correlations of yield contributing characters as well as the relative contributions of these characters on oil yield of Java citronella .

The existing base of variation in the clones of introduced slips has provided good clones for cultivation. Still, further upgrading is necessary for augmentation of oil yield. The upgrading is possible through genetic manipulation. The effectivity of any attempts for genetic manipulation depends on a clear understanding of the genetic background of the crop.

MATERIALS AND METHODS

Three cultivars were included in this experiment. Those were Java-I, Java-II and Guatemala. Inclusion of Burma was not possible due to paucity of propagules. Propagating materials were obtained by the courtesy of Bangalore Regional Centre of CIMAP.

This experiment was conducted in the Research Farm of BCKV at Kalyani, West Bengal. Information on soil of the Farm and the climate during growing period are furnished in Annexure I. Randomised Block Design with five replications was followed for this experiment.

Rooted slips or 'bibits' of about 20 cm length were transplanted on 5th March, 1980. Each slip with 1-2 tiller(s) was placed in one planting hole of about 12 cm depth, dug 60 cm apart on the side of the ridges at a distance of 75 cm. No manure or fertilizer was used. Weeding and irrigation were provided as and when needed. Harvesting was done in the first fortnight of October, 1980 at 5-6 leaf stage.

The biometrical data were recorded on freshly harvested individual clumps. Oil recovery and oil yield variables were determined from distillation by Clevenger's apparatus (1928).

For studies on variation and genetic parameters sixteen morpho-economic characters were included. These were as follows.

1. Oil yield from herbage in cc,
2. Oil yield from leaves in cc,
3. Oil yield from stems in cc,
4. Oil recovery from 100 g herbage in cc,
5. Oil recovery from 100 g leaves in cc,
6. Oil recovery from 100 g stems in cc,
7. Weight of herbage in g,

8. Weight of leaves in g,
9. Weight of stems in g,
10. Number of tillers,
11. Average number of leaves per tiller,
12. Average length of tillers in cm,
13. Average length of 3rd. leaf blades in cm,
14. Average maximum breadth of 3rd. leaf blades in cm,
15. Average length of stems in cm,
16. Average basal girth of stems in cm.

In citronella, only leaves are used for distillation (Guenther, 1950), since other parts contain lower percentages of oil and those are also of inferior quality (Singh et al., 1976; Virmani et al., 1979). For this reason, oil yield from leaf was taken as dependent variable and other five characters having bearing on this were selected as independent variables for studies on correlations and path-coefficient analysis. The independent characters included leaf weight, tiller number, tiller length, leaf length and leaf breadth.

Variance analysis for each variable was carried out to assess intervarietal variation and genetic parameters, viz., genotypic coefficient of variation (GCV), heritability in broad sense (H%) and genetic advance percentage of mean (GA% of mean).

Variance and covariance analysis for each of and for each pair of variables served for computing phenotypic, genotypic and environmental correlations from respective variance and covariance components. Path-coefficient analysis was carried out at phenotypic level following Dewey and Lu (1959). Details of the statistical procedures are furnished in Annexure - IV

RESULTS AND DISCUSSION

Intervarietal variation

The results on intervarietal variation are furnished in Table No. 1. Results of Analysis of variance are furnished in Annexure - V

These results have been compared with those from the experiment conducted at Bangalore Regional Centre of CIMAP by Kole et al. (1980). In the following discussion, that experiment is cited as experiment 'at Bangalore' for brevity.

In the present experiment, oil yield variables have registered significant variation at both 5% and 1% levels, while at Bangalore significant variation at 5% level was obtained for only oil yield from stem. Java-II has outyielded Java-I for stem and Guatemala with regard to herb, leaf and stem at both the levels. Java-I has been significantly (at 5% level) superior to Guatemala for oil yield from herb and oil yield from leaf.

Oil recovery from herb and from leaf have exhibited highly significant variation. At Bangalore, all the oil recovery variables registered highly significant variation. The difference between Java-II and Java-I has been insignificant but it was highly significant at Bangalore. Java-II has been consistently superior to Guatemala at both the levels in both the experiments except oil recovery from stem in the present experiment. The difference between Java-I and Guatemala has been insignificant in both the experiments for oil recovery from stem. For oil recovery from herb and from leaf Java-I has outyielded Guatemala at only 5% level, while at Bangalore, Guatemala surpassed Java-I at both the levels. Consistently higher

recovery of oil from fresh grass of Guatemala over 'Java' was reported by Loustalot and Fernandez Pol (1949). It appears that their 'Java' behaved like Java-I type of strain. For oil recovery variables also Java-II appears to be consistently superior.

All the three characters pertinent to weight have varied significantly at 5% level, whereas it was only stem weight at Bangalore to do so. Java-II significantly lagged behind Java-I and equalled Guatemala for stem weight at Bangalore. But Java-II has significantly outweighed Java-I for stem and Guatemala for stem, leaf and herb. Java-I has exhibited significant superiority over Guatemala for leaf weight only, while it significantly outweighed Guatemala for stem only at Bangalore. Poor performance of Guatemala as compared to 'Java' with respect to weight of fresh grass reported by Loustalot and Fernandez Pol (1949) finds support from the present experiment. This substantiates the earlier assumption that their 'Java' strain was of same behaviour as that of Java-I. In terms of weight variables also, Java-II, inso facto, emerges out as no less better than Java-I.

With regard to tiller number, variation realized has been highly significant. At Bangalore, variation was significant at only 5% level. Java-I has been found to significantly outnumber Java-II at both the levels and Guatemala at 5% level. But at Bangalore Java-I was significantly inferior to Java-II and did not differ from Guatemala. The results of the present experiment conform to the numerical observation of Ravikumar & Narayana (Personal Communication). They observed Java-I, Guatemala and Java-II to assume number of vegetative shoots as 90-110, 70-95 and 60-85 respectively.

Insignificant variation has been found in respect of leaf number. This finding is similar to that of the experiment at Bangalore.

Regarding tiller length Java-II and Java-I, with no significant difference in between, have surpassed Guatemala at both the levels. But at Bangalore Java-I was significantly superior to both Guatemala and Java-II, which had no difference in between.

With regard to leaf length the results have been similar to those for tiller length. At Bangalore Java-I was superior to both Guatemala and Java-II at both the levels and Guatemala was superior to Java-II at 5% level. Ravikumar and Narayana (Personal Communication) claimed Java-I to be tallest and they observed length of leaf blade of Java-I, Java-II and Guatemala as 85-110 cm, 60-75 cm and 60-70 cm respectively. Their findings are, more or less, similar to those obtained from the experiment conducted at the same place of experiment as theirs.

For leaf breadth Java-II has registered highly significant superiority over both Guatemala and Java-I and Guatemala has exhibited superiority over Java-I at 5% level. The variation for leaf breadth at Bangalore also was highly significant. But at both the levels, Java-II and Guatemala had no difference in between and both were better than Java-I at both the levels. Ravikumar and Narayana (Personal Communication) observed leaf breadth for Java-II, Guatemala and Java-I as 17-22 mm, 15-20 mm & 10-14 mm respectively. The findings of the experiment at Bangalore are, more or less, congruous to those of their numerical observation.

An insignificant variation has resulted for stem length. In contrast, a highly significant variation was observed at Bangalore, where at both the levels, Java-I was better than Guatemala, which, in turn, was better than Java-II.

Regarding basal girth at both the levels, Java-II has been found to be superior to Guatemala, which, in turn, has surpassed Java-I. At Bangalore at both the levels, Java-I was superior to both Java-II and Guatemala but the latter two strains did not differ.

The present experiment and the experiment conducted at Bangalore reveal comparable response for most of the characters. However, some dissimilarities have also cropped up. These may, presumably, have rooted from the completely contrasting and drastically different edapho-climates, interaction of which has a strong bearing upon the ultimate phenotypic expression of any quantitative character. Apart from environmental deviation, the intravarietal variations in all the cultivars at Bangalore seem to have played a vital role. The samples of 'bibits' collected for the present experiment were not systematic for the spectrum of variation for all the cultivars.

Another fact of interest emerges out of the experiment is that nine characters, viz., oil yield from stem, oil recovery from herb, oil recovery from leaf, stem weight, tiller number, tiller length, leaf length, leaf breadth and basal girth have exhibited consistently significant variation. But six characters, viz., oil yield from herb, oil yield from leaf, oil recovery from stem, herb weight, leaf weight and stem weight have revealed significant variation in either of the experiments. Leaf number has shown insignificant variation in both the experiments.

Java-II appears to be a consistently superior strain for most of the important characters. Moreover, it bears oil of good quality. Virmani *et al.* (1967b) reported superiority of Java-II over Java-I in terms of total aldehydes calculated as citronellal but a slight inferiority to Java-I regarding total alcohols calculated as geraniol. But superiority of Java-II over Java-I for both alcohols and aldehydes was claimed by Virmani *et al.* (1979). From repeated observations on Java-I, Java-II and Guatemala, Maryana *et al.* (1976) rated Java-II as the best type. Its oil also met the Indian Standard specifications (Anon, 1957) for Java type citronella oil of 85-97 per cent by weight of total alcohols (as geraniol) and 35-45 per cent by weight of total aldehyde (as citronellal). Its oil also gained acceptance from perfumery industry and, therefore, it was selected for large scale production. Ravikumar and Narayana (Personal Communication) found Java-II and Guatemala to meet Indian Standard specifications for Java type citronellal oil. But Java-II surpassed numerically both Guatemala and Java-I in terms of total alcohols calculated as geraniol and total aldehyde content calculated as citronellal. Slightly but consistently higher percentage of geraniol in oil of Guatemala over 'Java' was reported by Loustalot and Fernandez Pol (1949). Their findings find parity in those of Kole *et al.* (1980), who through GLC analysis observed Guatemala to lead with 26.026% geraniol. Guatemala was numerically better than Java-I (24.603%) but significantly superior to Java-II (20.179%) and Burma (17.908%) at both the levels. For citronellal percentage, Guatemala (45.01) was also found to be significantly better than Burma (35.188), Java-II (33.796) and Java-I (8.061) at both the levels. However, Java II was superior to Java-I at both the levels. Kaul

TABLE NO. 1

INTERVARIETAL VARIATION AND GENETIC PARAMETERS

CHARACTER	VARIETAL MEAN		GM	*F-VALUE	SEM	**CD5%	+++CD1%	H%	GCV	GA% OF MEAN
	JAVA-II	JAVA-I								
Oil yield from herb	12.766	10.704	6.588	10.8230**	0.9561	3.1179	4.8067	66.2686	29.9072	50.1515
Oil yield from leaf	9.628	8.632	4.896	10.6552**	0.7643	2.4923	3.8422	65.8824	30.7662	51.4426
Oil yield from stem	3.138	2.074	1.692	13.4445**	0.2039	0.6664	1.0274	71.3377	31.3266	54.5040
Oil recovery from herb	0.470	0.414	0.332	12.9393**	0.0192	0.0611	0.0942	70.4828	16.4556	28.4481
Oil recovery from leaf	0.738	0.646	0.498	12.7505**	0.0339	0.1107	0.1706	70.1502	17.3933	31.1813
Oil recovery from stem	0.198	0.180	0.164	3.0692 ^I	0.0097	0.0316	0.0487	29.2710	7.7320	8.6331
Herb weight	2826.830	2495.933	2030.792	5.9347*	163.7584	535.3257	825.2744	49.6716	14.8765	21.5983
Leaf weight	1250.599	1350.407	967.305	6.9922*	75.1471	245.1050	377.8613	54.5132	15.4180	23.3726
Stem weight	1576.231	1145.529	1063.489	8.4371*	94.7812	309.2240	476.7091	59.7976	20.4940	32.6464
Tiller number	117.534	175.566	125.150	9.3971**	10.2630	33.5505	51.7224	62.6783	21.3831	34.8735
Leaf number	6.108	5.408	5.822	4.0286 ^I	0.1742	0.5719	0.8816	37.7225	5.2800	6.6807
Tiller length	164.6877	160.8018	138.5338	47.2935**	2.0521	6.6922	10.3170	90.2522	8.9805	17.5639
Leaf length	117.150	121.906	99.080	29.2279**	2.2277	7.2648	11.1997	84.9524	10.4644	19.8536
Leaf breadth	2.326	1.440	1.554	311.7152**	0.0273	0.0892	0.1376	98.4163	27.1385	55.4710
Stem length	33.408	33.022	33.672	0.2572 ^I	0.6694	2.1829	3.3652	17.4483	1.7289	1.4877
Basal girth	3.220	2.224	2.618	162.3984**	0.0394	0.1284	0.1979	96.9952	18.6083	37.7516

+ Ft 5% = 4.46, Ft 1% = 8.65, ** Significant at 1% level, * Significant at 5% level,

et al. (1977) also evaluated the oils of 'Java' (Java-II is called as 'Java', Virmani et al., 1979) and 'Burmese' by GLC analysis. They found much higher percentage of citronellal in 'Burmese' (66.3%) than in 'Java' (48.6%). But 'Java' was found to be superior in respect of citronellol (14.2%) and geraniol (29.6%). 'Burmese' contained 6.4% citronellol and 14.7% geraniol. According to Guenther (1968) GLC standard of Java citronella oil for citronellal, geraniol and citronellol is as follows.

Citronellal	- 32-45%,	best quality	above 38%
Geraniol	- 12-18%,	" "	" 16%
Citronellol	- 11-15%,	" "	" 12%

So, oil of Java-II fairly meets the requirements of GLC standard also. Even the oil exceeded the specifications for best quality for geraniol at Bangalore and the oil of 'Java' exceeded the requirements for best quality for citronellal, citronellol and geraniol of GLC standard.

So, on the basis of encouraging overall performance regarding most of the morpho-economic characters, Java-II deserves to be a potential base material for genetic amelioration of this crop for yield and quality.

Genetic Parameters

The estimates of the genetic parameters under study, viz., GCV, H% and GA% of mean for 16 characters are furnished in Table No. 1. The values realised for GCV and GA% of mean have been arbitrarily rated as low (below highest value/3), moderate (within highest value/3 to 2 x highest value/3) and high (exceeding 2 x highest value/3). The same assumption with 100 as the highest value has been adopted for rating H%.

High GCV estimates have been obtained for oil yield from stem, leaf and from herb, leaf breadth and tiller number. Moderate values have been obtained for stem weight, basal girth, oil recovery from leaf and herb, leaf weight, herb weight and leaf length. Low have been the estimates for tiller length, oil recovery from stem, leaf number and stem length.

So far as the values of GA% of mean are concerned, the characters under high category are leaf breadth, oil yield from stem, leaf and from herb and basal girth. Moderate values have been assumed by tiller number, stem weight, oil recovery from leaf and from herb, leaf weight, herb weight and leaf length. Low values are associated with tiller length, oil recovery from stem, leaf number and stem length.

High H% estimates have been obtained for leaf breadth, basal girth, tiller length, leaf length, oil yield from stem, oil recovery from herb and from leaf. Moderate values have been realised for oil yield from herb and from leaf, tiller number, stem weight, leaf weight, herb weight and leaf number. Low are the values associated with oil recovery from stem and stem length.

It is of interest to note a very close behaviour of GCV and GA% of mean. For each character, value is high, moderate or low for both the parameters. Even the sequential positions of characters in order of GCV estimates are, more or less, same while compared to those arranged in order of GA% of mean and vice versa. But such type of similarity of H% with either GCV or GA% of mean is beyond generalisation.

Out of 16 characters, leaf breadth emerges out as most promising. It has registered highest values for H% and GA% of mean and has ranked

4th for GCV value. An association of high values of $H\%$, GCV and $GA\%$ of mean proves its worth as the most important selection criterion. Since, this type of association indicates additive gene effects and leads to consequent genetic gain from selection (Panse, 1957).

An association of high estimates of $H\%$, GCV and $GA\%$ of mean has been realised for oil yield from stem also. For the other two oil yield variables an association of high GCV and $GA\%$ of mean and moderate $H\%$ has been obtained. In contrast, oil recovery from herb and from leaf have registered an association of high $H\%$ and moderate GCV and $GA\%$ of mean. However, the oil yield variables are complex characters. Oil yield variables as well as oil recovery variables can not be considered as selection criteria, since assessment of these variables by distillation for a large number of clumps in any breeding experiment is not practically feasible.

Basal girth has assumed high values for $H\%$ and $GA\%$ of mean and moderate estimate for GCV. This character may be based upon in selection.

All the three weight variables have registered moderate estimates for GCV, $GA\%$ of mean and $H\%$. These characters are difficult to handle in field experiments and may be considered when sample size is small.

High GCV coupled with moderate $H\%$ and $GA\%$ of mean have been realised by tiller number. Leaf length has assumed high $H\%$ together with moderate GCV and $GA\%$ of mean. These two characters may also be considered in selection.

Despite low GCV and $GA\%$ of mean, tiller length has registered high $H\%$, for which this character deserves consideration.

The genetic parameters of the three characters, viz., oil recovery from stem, leaf number and stem length, which have exhibited insignificant variation also, reveal unaccountable estimates. Both oil recovery from stem and stem length have been of a combination of low GCV, low GA% of mean coupled with low H%. On the other hand, leaf number has assumed low GCV, low GA% of mean associated with moderate H%.

From the above discussion, leaf breadth appears to be the most important character as a selection criterion. Basal girth, leaf length and tiller number can also be taken in order of preference as selection criteria. Tiller length and weight variables may be included on consideration.

Correlations and Path-coefficient analysis

Direct, phenotypic, genotypic and environmental correlation values of five characters inter se and with oil yield from leaf, hereinafter cited as oil yield, are presented in Table No. 2. The results of path-coefficient analysis (at phenotypic level) for those characters are presented in Table No. 3.

It is evident from Table No. 2 that the correlations at phenotypic and genotypic level in each cell are of same direction and have assumed close values. The estimates of direct and phenotypic associations are also remarkably close enough in magnitude and are of same sign. Most interestingly, the relationship of phenotypic, genotypic and environmental correlation estimates along with heritability values for a pair of characters universally followed a general formula as follows :

$$P = G \sqrt{H_1 H_2} + E \sqrt{(1-H_1)(1-H_2)}$$

where P, G and E are phenotypic, genotypic and environmental

correlations estimated as usual from respective variance and covariance components; H_1 and H_2 are heritability (in broad sense, $H\%$) values of the two characters involved in association. The values of $\sqrt{H_1 H_2}$ and $\sqrt{(1-H_1)(1-H_2)}$ and $H\%$ are also tabulated (Table No.2)

The associations at environmental level between leaf weight and tiller length, leaf weight and leaf breadth, tiller number and tiller length, tiller number and leaf length, tiller number and leaf breadth and between leaf breadth and oil yield have been found to be negative, whereas phenotypic and genotypic correlations have been of same direction. For each of these pairs P/G is less than $\sqrt{H_1 H_2}$.

Secondly, the phenotypic correlation value has exceeded its genotypic counterpart for associations between leaf weight and tiller number and tiller number and oil yield. In both the cases E/G is more than $(1 - \sqrt{H_1 H_2}) / \sqrt{(1-H_1)(1-H_2)}$.

In rest of the cases phenotypic correlation values have been less than genotypic values, and in each of these cases E/G is less than $(1 - \sqrt{H_1 H_2}) / \sqrt{(1-H_1)(1-H_2)}$.

This relationship was deduced by Searle (1961) to study the relationship of phenotypic, 'genetic' and environmental correlations along with heritability values derived from certain animal records. The present findings indicate that this relationship is as good in Java citronella also. Substitution of phenotypic correlation estimate by direct correlation value also satisfies the equation.

Path-coefficient analysis at phenotypic level has revealed

positive direct contribution of leaf length, leaf breadth, tiller number and leaf weight on oil yield. But tiller length has registered a negative direct effect.

Leaf length has exhibited highest positive contribution. But its indirect effect via tiller length has been very high and negative. This effect has outweighed its low but positive indirect effects via tiller number and leaf breadth. But indeed a very high direct effect, even reduced to some extent, has resulted in a high positive association with oil yield. The positive indirect contributions of other characters via leaf length, particularly very high effects of leaf weight and tiller length and moderate effect of tiller number are also encouraging. The leaf length has been involved in a high positive association with leaf weight. Correlation value of this character with tiller length was also positive and very high.

Leaf breadth is second in magnitude of direct contribution on oil yield. But its total indirect effect has been negative and has assumed a high value. As a result the total effect has been diminished to a moderate value in spite of a very high direct effect. It has registered moderate negative indirect effect through tiller number and tiller length. On the other hand, tiller number has exhibited a negative and high indirect effect via leaf breadth. This indirect effect has largely counterbalanced the positive indirect effects mainly a moderate effect of tiller length and a low but positive effect of leaf length via leaf breadth. This relation between leaf breadth and tiller number is explicit in their high negative association.

A high positive direct effect on oil yield has been manifested

TABLE NO. 3

PATH-COEFFICIENT ANALYSIS OF PHENOTYPIC CORRELATIONS

CHARACTER	DIRECT EFFECT	INDIRECT EFFECT VIA				TOTAL INDIRECT EFFECT	TOTAL EFFECT	PHENOTYPIC COEFFICIENT - RELATION WITH OIL YIELD	GENOTYPIC COEFFICIENT - CORRELATION WITH OIL YIELD
		Leaf weight	Tiller number	Tiller length	Leaf breadth				
Leaf weight	0.0616	-	0.4618	-0.6720	0.8024	0.1062	0.7984	0.860	0.915
Tiller number	0.6523	0.0436	-	-0.1475	0.4903	-0.6766	-0.2902	0.362	0.137
Tiller length	-0.9767	0.0424	0.0985	-	1.0927	0.5681	1.8017	0.825	1.057
Leaf length	1.1674	0.0476	0.2740	-0.9142	-	0.2203	-0.3723	0.795	0.965
Leaf breadth	1.1153	0.0059	-0.3946	-0.4962	0.2300	-	-0.5549	0.468	0.604
RESIDUAL EFFECT									
							0.2553		

by tiller number. Its low but negative total indirect effect has resulted mainly due to a high negative effect of leaf breadth which has surpassed a moderate positive effect of leaf length. The total effect, thus, has been reduced to only a moderate value. The total indirect effect through tiller number has been moderate resulted mainly from outweighing of the moderate negative effect of leaf breadth by moderate positive effect of leaf weight and low but positive effect of leaf length. It has been involved in a very high positive association with leaf weight. Its association with leaf breadth has already been delineated.

Although a minimum but positive direct effect has been realised for leaf weight, a high total indirect effect has solely yielded in a very high positive association with oil yield. A very high indirect effect through leaf length substantiated by the moderate effect via tiller number has surpassed by far the negative high effect through tiller length. The indirect effects through leaf weight have assumed only negligible values.

Tiller length has been the only character to register a very high negative direct contribution on oil yield. The indirect effects of other characters via this one are also negative. But its indirect contribution has been very high through leaf length and moderate through leaf breadth. These two indirect effects have contributed for an strikingly high total indirect effect which has by far outweighed the very high negative effect to pave the way for a very high positive correlation with oil yield. Its correlation with leaf length has already been discussed.

From the erstwhile discussion, leaf length and leaf breadth

emerge out as the most promising characters as selection criteria. Tiller number also deserves inclusion along with those. Leaf weight may be considered for its high indirect effect, while tiller length may not be taken into consideration. These findings, in fact, confirm the earlier prediction on identification of selection criteria on the basis of genetic parameters.

Component approach in selection is gaining importance day by day. Grafius (1959) opined that yield in barley is an artifact, since genes for yield per se were not found to exist. The same author (1956) defined yield in oats as a geometrical construct, viz., a parallelopiped with the yield components as the edges.

It was worthwhile a suggestion by Nickell & Grafius (1969) to partition the more complex quantitative traits like yield into simpler components to have some insight into genotype \times environment interactions. Certain complex characters including yield may be the product of actions and interactions of their components. Observations of the components, according to them, will sometimes furnish a clue as to why the complex trait fails to respond in an expected manner. They also viewed that selection for yield while ignoring the components may produce some unusual and undesirable results and the converse was also thought to be true.

Yield was described to be the product of its component traits in various crops by Sinclote (1947), Robinson et al. (1951), Johnson et al. (1955), Grafius (1956, 1959), Dewey & Lu (1959), Nickell & Grafius (1969) and several other workers.

SUMMARY AND CONCLUSION

(Three cultivars of Java Citronella, Cymbopogon winterianus Jowitt, have been tested for their performance in the Gangetic alluvium Farm land of BCKV.)

On the basis of 16 morpho-economic characters Java-II has been found to give the best response. The varieties show significant difference with respect to all characters except oil recovery from stem, leaf number and stem length.

(Estimates of genetic parameters of 16 characters indicate that leaf breadth can be considered to be reliable criterion for clonal selection. Basal girth, leaf length and tiller number are the next best characters in order of merit, followed by tiller length and the weight variables which can be taken into consideration collectively. Path-coefficient analysis has revealed leaf length and leaf breadth followed by tiller number to be the most promising characters. Leaf weight also deserves consideration.)

(Java-II has emerged as the best cultivar on the basis of most of the morpho-economic traits.) Character analysis revealed statistically significant variation in oil yield variables, oil recovery from herb and leaf, weight variables, tiller number, tiller length, leaf breadth and basal girth. Oil recovery of stem, leaf number and stem length have registered insignificant variation. On the basis of genetic parameters, viz., GCV, GA% of mean and H%, leaf breadth has appeared to be most promising as a selection criterion. Basal girth, leaf length and tiller number have appeared to be appreciably profitable in selection. Tiller length and the weight

variables may also deserve consideration. (Studies) on correlations and path-coefficient analysis with leaf weight, tiller number, tiller length, leaf length and leaf breadth as independent variables and oil yield from leaf as dependent variable, (have indicated leaf length, leaf breadth followed by tiller number to be important characters in selection programme for higher oil yield.)

FUTURE SCOPE OF WORK

1. Performance of Java-II may be tested in 'multi-locations' of this state particularly including the problem zones, such as, drought prone areas, saline tracts and acid soils of terai belts.
2. Application of "discriminant function" (Smith, 1936) in selection is necessary to find out relative weightages to be conferred on various selection criteria to achieve maximum genetic gain.
3. Attempts of combining the desirable characters of the cultivars may be made in the agro-climates where this crop flowers and set viable seeds.
4. Java-II may be selected as base material for mutation breeding works with a view to creating variation and selecting desirable genotypes, if any.

SECTION - II

CHARACTER ANALYSIS ON

X-IRRADIATED POPULATION OF JAVA CITRONELLA

(Cymbopogon winterianus Jovitt)

INTRODUCTION

As already been delineated, Java-II, the superior strain amongst the four cultivars of Java Citronella, C. winterianus, is exclusively cultivated in different zones of India. Though this crop is highly remunerative, the demand for its product has been met through extension of area rather than effective augmentation of productivity. In fact, the attempts to increase the quantity and quality of oil have been meagre considering the needs (Chauhan et al., 1976; Choudhary and Kaul, 1980a).

The present author observed low intravarietal variation for most of the agronomic characters in this crop (Kole et al., 1980). Choudhary and Kaul (1980a) also reckoned the natural (within cultivar) variability of almost all the agronomic characters worthwhile to be considered in any breeding scheme in this crop to be very narrow.

Hence, improvement of this crop for oil yield desiderates induction of variability by hybridization or mutation. The scope of employing cross breeding is limited due to certain anomalies pertinent to flowering and seed setting, particularly in the Gangetic alluvial tracts of West Bengal. Although free interspecific and intraspecific hybridization are reportedly prevalent in the genus Cymbopogon Spreng. (Anon, 1950; Bor, 1953; Gupta, 1971) sporadic flowering had been reported in this species (Anon, l.c.). Ravikumar and Narayana (Personal Communication) and Ravikumar et al. (1977) observed sporadic flowering in Guatemala but profuse flowering in Java-I and Java-II. Ravikumar et al. (l.c.) recovered very few viable seeds in Java-I, but Java-II and Guatemala did not set any seed. Bor (1960) also reported the occurrence of partial sterility. But Chandra (1973) observed Java-II to flower

and to produce seeds. Observations at West Bengal plains has revealed no flowering in Java-II and Guatemala either. However, malformation was noted in the inflorescence shoots, which emerged only very sporadically, in Java-I. Virmani et al. (1979) reported profuse flowering of Java citronella in South India and at higher altitudes in the hills of North-Eastern India, but only sporadic flowering in the plains of North and North-Eastern India. They also reported failure to form viable seeds because of meiotic irregularities. All these lead but to the conclusion that hybridization will not be a feasible method for genetic manipulation in Java citronella.

Utilization of physical and chemical mutagens is a worthwhile proposition in this crop. Vegetative propagation, a rule in cultivation of this crop, is conducive to effective mutation breeding. As revealed from the available literature with the author, the use of mutagens in improvement of vegetatively propagated crops is not insignificant. Only the list compiled by Sigurbjornson and Micke (1973) of the varieties of vegetatively propagated plants developed by utilizing induced mutations and the bibliography prepared by Nybom and Micke (1973) on mutation breeding of vegetatively propagated plants and woody perennials will prove the worth of mutation breeding as a tool in improvement of vegetatively propagated plants.

Gupta (1973), Chauhan et al. (1976), Atal (1979) and Choudhary and Kaul (1980a) suggested the use of physical and chemical mutagens to induce variability in this crop. Chauhan et al. (l.c.) isolated a few promising plants with increased tillering, number and size of leaves and thereby higher herb yield by use of alkylating mutagens,

viz., DES, DMS and DMSO. A strain, "RRLJCR-3-1970" was also developed through EMS treatment (Ganguly et al., 1979).

So far as the physical mutagens are concerned, occurrence of morphological mutants in the gamma ray-exposed population was reported by Chandra (1973). With X-ray treatment Choudhary and Kaul (1980a) were successful to induce variability in plant height and fresh herbage yield but the variability for the latter character was negatively skewed. Reduction in various quantitative characters in their experiment was similar to that reported by Chandra and Gupta (1975) who had used gamma rays.

But effective use of ionizing radiations, viz., X-rays and gamma rays, in other species of Cymbopogon Spreng. aiming at inducing morphological stimulation, isolation of positive variants and rectification of defects in oil quality are quite encouraging (Kapoor and Datta, 1967; Gupta, 1969a; Gupta and Kapoor, 1970; Choudhary et al., 1976; Kaul et al., 1978; Atal, 1979; Nair, 1979, 1980; Choudhary and Kaul, 1980a; Verma et al., 1980).

Mutagenesis of large botanical material, which constitutes the basic propagule in a vegetatively propagated crop, calls for a different approach of selection. Dechimerisation of the treated propagules should be the first step to identify the extent of variation. The means of agronomic characters would naturally be reduced due to induced deleterious effects on growth of most of the axillary buds. This does not rule out the possibility of having a few of the buds with positive effect.

Therefore, assay of characters of economic importance from

clones (VM₂) raised from every axillary bud developing from a treated propagule (VM₁) into a plant is essential. (The present study is designed to delineate the extent of genetic divergence induced in some of the agronomic characters by X-ray treatment as well as to identify superior genotypes with reference to the three important yield contributing characters, viz., leaf length, leaf breadth and tiller number and finally in terms of leaf weight. The yield and composition of oil were assayed for some of the selected clumps.)

REVIEW OF LITERATURE

As indicated in the earlier section, improvement in Java citronella was sought mainly through selection from clones. The estimates of intervarietal differences reveal the existence of appreciable discriminability for isolation of improved genotypes. In fact, within the limit of the existing genetic resource the base of variation is not wide enough for developing breeding programme for improvement of oil yield.

Reports on induction of variation through mutagenesis in C. winterianus are meagre. Chandra (1973) cited the recovery of large number of leaf mutation ranging from pure white to striped green and yellow. He obtained a few plants with changed morphological features and some plants with elongated stem. These modifications were obtained by using gamma irradiation. Chandra and Gupta (1975) reported the reduction of various quantitative characters due to gamma irradiation.

Choudhary and Kaul (1980a) treated dormant vegetative slips with 5 kR, 7.5 kR and 10 kR X-rays (at the dose rate of 770 R/min). They observed a gradual decrease in survival percentage and tiller number with increase in dose. Plant height and leaf length were also adversely affected, though reduction in 7.5 kR treatment was less compared to 5 kR treatment. Fresh herbage yield was also reduced due to treatment, whereas oil percentage remained unaffected. They computed LD₅₀ for dormant vegetative slips treated with X-rays to be 8.5 kR. They further noted an increase in variability for plant height due to treatment. Induced variability for fresh herbage yield per plant at 7.5 kR treatment was negatively skewed.

Chauhan et al. (1976) isolated a few promising plants of Java

citronella through treatment of slips with 0.5% solution of DES, DMS and DMSO. The improvement was mainly due to higher herb yield which was the manifestation of enhanced tillering, increased leaf number and bigger leaf size. Oil quality of the selected desirable plants did not, however, differ appreciably from that of the control. Ganguly *et al.* (1979) also isolated a strain, 'RELJON-3-1970', through EMS treatment of slips.

Though the few reports on the use of physical mutagenesis as a tool for improvement of Java citronella clones are not so encouraging, some results obtained with other allied species of Cymbopogon Spreng. reveal the turn over of some desirable characters for effective clonal selection. Kapoor and Datta (1967) exposed dormant vegetative slips of Ceylon citronella, C. nardus to 2000-Curie-gamma-cell irradiation for 1,2,5,10,17.5 and 25 hours. Characteristically enough, the mean number of tillers per plant increased with the increase of duration of treatment. Almost same was the fate for the mean length of third leaf except at 25 hours. Mean height of plant remained unaffected compared to control at 2 and 5 hours duration of treatments. In all other treatments mean plant height was lower than the control. In accordance with their report the survivability per cent of axillary buds indicated as germination percentage decreased gradually with the increase of duration of treatment. LD₅₀ for survivability appeared between 1 and 2 hours duration of treatments.

Choudhary *et al.* (1976) found that 5 and 10 kR X-ray treatments (770 R/min) of strains RRL-14 (C. khasianus), RRL-16 (C. pendulus) and RRL-59 (C. flexuosus) provided good opportunity for selection, preferably with reference to improvement of one or more components of essential

oil. Selection in the treated vegetative generation, referred to as M_1 generation of RRL-59 resulted in the identification of a mutant with decreased methyl-eugenol component, which is regarded to contribute to the improvement of the quality of the essential oil (Kaul *et al.*, 1978; Atal, 1979; Choudhary and Kaul, 1980b). On the other hand, Gupta (1976-'77) found that irradiation of *C. pendulus* vegetative slips with 6, 8 and 10 K rad of gamma rays had significantly decreased the mean values for survival, plant height, leaf and tiller number per plant and leaf size in the treated vegetative generation compared to those of the control. Nair (1980) reported improvement in grass yield per plant and augmentation in essential oil yield to the extent of 0.42% as against 0.23% in the standard type through gamma ray treatment of caryopses of *C. flexuosus* var. OD-19 followed by selection in the clonal progenies of plants raised from treated seeds.

Gupta (1969a) and Gupta and Kapoor (1970) used Co^{60} source for acute irradiation of *C. martinii* seeds (10-14% moisture) with 20, 30, 40 and 50 K rad doses of gamma rays. Germination and survivality per cent gradually decreased with increase of doses. LD_{50} for germination was estimated to be 35.5 K rad. Compared to control, growth at lower doses was vigorous in the treated generation. The mean values for plant height, length and breadth of flag leaf were higher than those in the control in the M_2 generation also. Verma *et al.* (1980) used low doses of gamma rays to dry seeds of *C. martinii* var. *notia*. The doses used by them were 5, 7, 10 and 15 kR gamma rays. They achieved success in isolating 5 variants with superior morphological characters.

The review of available literature reveals reports not quite in agreement with respect to induced modification of vegetative

characters and essential oil yield. In all cases with the vegetatively propagated species records were taken from the treated vegetative generation. The fact that irradiation of the vegetative slip had involved a large plant part usually with more than one axillary bud having least chance of uniform effect of irradiation, was not taken into serious consideration, when the metrical data, slip-wise and treatment-wise, were averaged to compare with the performance of the untreated control. In spite of that the trends of deviation in mean values towards positive or negative side compared to control do indicate that a proper evaluation of the treated clones after dechlorination in the VM_1 generation would lead to appropriate assay of induced variation. The present investigation, therefore, has been found pertinent with respect to identification of improved genotypes of Java citronella from a broader base of genetic divergence than found in the natural resource.

MATERIALS AND METHODS

Genetic material

The 'bibits' were collected from a clump of a strain, KS-CW-S-1, developed by selection from Java-II and tested against Java-I, Guatemala and its parent under two years' primary performance trial at Kalyani agro-climate of West Bengal.

Preparation of 'bibits'

Mature and more or less uniform vegetative slips of on an average 15 cm length were collected from a one year and three and a half months' old clump. The slips were trimmed of their roots and were cleaned of old and dry leaf sheaths.

Irradiation

5 groups, each consisting of 12 'bibits', were irradiated with 5 doses, 3 kR, 6 kR, 9 kR, 12 kR and 15 kR of X-rays (100 KV, 11 mA). One set with 12 'bibits' was kept as control. Irradiation was done after 5 hours of preparation of 'bibits'.

Transplanting and cultural practices

Transplanting was done after 3 hours of irradiation on 19th June, 1981 in another subplot of the same experimental plot of the earlier experiment (Section - I). Planting and cultural methods were same as the earlier experiment. Harvesting was done in the first fortnight of February, 1982, at around 5 leaf stage of the control. The climatic conditions for the growing period are presented in Annexure-II.

Recording of data on VM₁

Counts for survival were taken at 15, 21 and 30 days after transplanting. Final survival was recorded during harvest.

(within $\mu \pm SE$), positive variant (exceeding $\mu + SE$) and negative variant (below $\mu - SE$) for each character on the basis of mean and standard error of the respective control. The positive variants for leaf length, leaf breadth and tiller number were isolated as 'promising clumps'. Positive variants for one or two of these three characters and all other variants were kept beyond the perview of the present investigation. Negative variants for all the five characters were marked as 'subvitals'. These were kept for further studies.

Data on leaf weight were taken on the promising clumps. The positive variants for leaf weight amongst the promising clumps were isolated as 'selected clumps'. Oil recovery from leaf was determined on some of the selected clumps taken randomly. Oil composition was evaluated by Gas-liquid chromatography (GLC) for some randomly taken selected clumps of three families, one from each of 3, 6 and 9 kR treatments. Gas-liquid chromatography (GLC) of oil was carried out on a PYE SERIES 104 CHROMATOGRAPH (PYE UNICAM) using SE-30 (10%) fitted with stainless steel column, 2 m long and 2 mm diameter and fitted with a FID detector. Nitrogen was used as the carrier gas with a flow rate of 20 ml/min. Column temperature had been 100-200°C with an isothermal temperature at 100°C for 9 min, thereafter, programmed at 10°C/min increment rate upto 200°C. Injection port and detection temperatures were 200°C and 250°C respectively. Chart speed was 320 mm/hr. The peaks were identified by comparison of their relative retention times and also by peak enrichment technique.

Statistical

Range (R), mean (μ), standard error (SE) and coefficient of variation (CV) were estimated as usual.

RESULTS AND DISCUSSION

OBSERVATIONS ON VM₁ GENERATION

Survivality of irradiated 'bibits'

Table No. 4 shows the survivality of treated 'bibits' in terms of emergence of aerial shoots from axillary buds. It is evident from the table that LD₁₀₀ has appeared at 12 kR, while LD₅₀ for survivality has been recorded at 3 kR considering the records of survival at harvest. The comparatively high percentage of survivality at 9 kR vis-a-vis at 6 kR is not explainable.

TABLE NO. 4

SURVIVALITY OF IRRADIATED 'BIBITS'

DOSE IN kR	DATA RECORDED AT					HARVEST	
	INITIAL PLANTING	14 DAT	21 DAT	28 DAT	35 DAT		% OF CONTROL
0	12	11	11	10	10	10	100
3	12	6	6	5	5	5	50
6	12	7	5	1	1	1	10
9	12	7	7	5	5	5	50
12	12	3	3	1	0	0	0
15	12	6	6	0	0	0	0

Decrease of survival of irradiated slips due to increase of dose of ionizing radiations was reported by Choudhary and Kaul (1980a) in C. winterianus (X-ray), Kapoor and Datta (1967) in C. nardus (gamma ray), Gupta (1976-'77) in C. pendulus (gamma ray) and by Choudhary et al. (1976) in C. khasianus, C. pendulus and C. flexuosus (X-ray).

Choudhary and Kaul (1980a) recorded LD₅₀ for survival to be 8.5 kR X-rays for C. winterianus. They observed a gradual decrease in survivability with the increase of X-ray doses. The 50% survival at 9 kR in the present experiment may be regarded as to corroborate Choudhary and Kaul's (1980a) observations. It appears that the drastic reduction of survivability, particularly due to 6 kR treatment may be for causes other than treatment alone.

Changes in agronomic characters

Records on VM₁ (Table No. 5) indicate that irradiation has adversely affected leaf length, tiller length and leaf breadth at all the doses. Out of these three characters leaf length exhibits appreciable decrease in mean values at all three doses and tiller length shows such decrease at 3 kR and 9 kR. Tiller number has been highly affected at 3 kR but it exhibits a slight increase at 9 kR and a spectacular increase due to 6 kR dose of irradiation. Irradiation has exhibited a positive effect on leaf number which shows a high increase at 6 kR and almost no change at 3 and 9 kR. In general, adverse effects are most at 3 kR followed by 9 kR and 6 kR in that order. These reveal that the consequences of irradiation, either increase or decrease of mean values of the characters, do not have any direct relation with the increase in dose.

Chandra and Gupta (1975) reported the reduction in various quantitative characters due to gamma irradiation of the same species. Choudhary and Kaul (1980a) observed adverse effects of X-rays on plant height, leaf length, leaf width, tiller number and fresh herbage yield. They also found the mean values for plant height, leaf length and fresh herbage yield to be more in 7.5 kR than 5 kR. The mean values,

however, decreased again at 10 kR dose. So, the decrease in mean values had no direct relation with the increase in dose for these three characters.

Chandra (1973) isolated some mutants with elongated stem by using gamma irradiation in C. winterianus. Present author is not aware of any information, except that in the erstwhile report, of morphological stimulation of agronomic character due to irradiation in this species. But in the closely related species, C. nardus, Kapoor and Datta (1967) obtained increased mean number of tillers and mean length of third leaf due to irradiation of vegetative slips with gamma rays. The increase in these two characters was not exactly corresponding to the increase of exposure.

Gupta (1976-'77) reported significant decrease of morphological characters with the increase of exposure to gamma irradiation of slips of C. pendulus. Choudhary et al. (1976) also reported the reduction of plant height with the increase of dose of X-rays to vegetative slips of C. khasianus, C. pendulus and C. flexuosus.

The results also indicate the increase of variation, in terms of coefficient of variation, due to irradiation at 3 kR and 9 kR doses in all the characters except leaf number at 3 kR. However, increase of coefficient of variation for leaf breadth at 3 kR and for leaf number at 9 kR is low. The variation for 6 kR treatment, however, could not be computed because of recovery of only one clump after irradiation.

The estimates for range also indicate increase in all cases except leaf breadth at 3 kR and leaf number at both 3 and 9 kR.

Except in these three cases, irradiation has generally induced variation.

The increase of variation in the characters, particularly leaf length, leaf breadth and tiller number, indicates the chance of isolating some desirable clumps in the dechimerized population at VM₂ generation. Induction of variability with respect to plant height and fresh herbage yield per plant due to X-ray treatment of vegetative slips of C. winterianus was reported by Choudhary and Kaul (1980a) also.

OBSERVATION ON VM₂ GENERATION

Initial screening for hardiness

From Table No. 6, it is of interest to note that one family from 3 kR dose, the only family from 6 kR dose and two families from 9 kR treatment have exceeded the control with regard to the number of 'bibits' escaping the minimal cultivation followed at the nursery for screening of hardy genotypes. The survivability of the family of 6 kR is indeed spectacular. Those of the two families of 9 kR are appreciably high. However, the dose-wise averages of survival records for 3 kR and 9 kR are below the control. Similar results have been obtained during final planting and also during harvest barring the family of 3 kR dose which is below the control.

Considering final planting the survival at harvest is equally high in the control and the VM₂ families except one from 3 kR which shows only 55.56% survivability compared to 95.45% of the control. Two families of 3 kR, the only family of 6 kR and three families of 9 kR dose have exceeded the survival percentage of the control.

Observations on agronomic characters

The estimates of range, coefficient of variation, standard error and mean of 11 VM₂ families and the respective controls are

TABLE NO. 6

SURVIVAL RECORD AT VM₂ GENERATION

VM ₂ FAMILIES	INITIAL PLANTING	AFTER SCREENING	% OF INITIAL PLANTING	DURING INITIAL PLANTING	DURING HARVEST	% OF FINAL PLANTING	% OF INITIAL PLANTING
0 KR (CONTROL)	250	124	49.60	110	44.00	105	42.00
3 KR P ₁	64	8	12.50	6	9.38	5	7.81
P ₂	92	14	15.22	10	10.87	10	10.87
P ₃	81	17	20.99	10	12.35	9	11.11
P ₄	20	10	50.00	9	45.00	5	25.00
P ₅	155	30	19.35	24	15.48	24	15.48
TOTAL	412	79	19.17	59	14.32	53	12.86
6 KR P ₁	193	177	91.71	170	88.08	169	87.56
9 KR P ₁	173	34	19.65	20	11.56	17	9.83
P ₂	89	61	68.54	58	65.17	55	61.80
P ₃	114	71	62.28	67	58.77	65	57.02
P ₄	59	26	44.07	22	37.29	22	37.29
P ₅	105	49	46.67	41	39.05	41	39.05
TOTAL	540	241	44.63	208	38.52	200	37.04

shown in Annexure - III. The deviations of range, coefficient of variation and mean of the VM₂ families are presented in Table No. 7.

The dose-wise averages on deviations of mean indicate very little change whether in positive or in negative direction. For 3 kR, all the five characters studied have registered negative shift. For 6 kR, leaf length, leaf breadth and tiller length show positive deviation, whereas tiller number and leaf number exhibit negative shift. It should be mentioned here that the trend of deviation has been just opposite in the VM₁ of 6 kR treatment. In case of 9 kR, positive shift has been found for leaf length, tiller number and tiller length while leaf breadth and leaf number have shifted negatively.

So far as the individual families of 3 kR (i.e. P₁ to P₅) are concerned, shift is negative in most of the cases. P₁ exhibits negative shift for all the characters except leaf number. However, deviation for all characters is low. A negative shift has been realised for all the characters in P₂ and P₃. But deviation for tiller number in P₂ and leaf length, tiller number and tiller length in P₃ is high. P₄ manifests high positive shift for tiller number, high negative shift for leaf length and leaf breadth and low negative deviation for leaf number and tiller length. In P₅ tiller number and leaf number show negative shift and unique to this family leaf length, leaf breadth and tiller length exhibit positive shift. A positive shift of leaf length and leaf breadth explicitly proves this family to be promising. The estimates for leaf length, tiller length and tiller number are high.

In the progenies of 9 kR dose, in P₁, low positive shift has been observed for tiller number and leaf number while deviation has

TABLE NO. 7
 DEVIATION OF AGRONOMIC CHARACTERS AT VM₂ GENERATION (IN % OF CONTROLS)

FAMILY-WISE ESTIMATES										DOSE-WISE AVERAGED ESTIMATES										
P ₁		P ₂		P ₃		P ₄		P ₅		P ₁		P ₂		P ₃		P ₄		P ₅		
R	CV	R	CV	R	CV	R	CV	R	CV	R	CV	R	CV	R	CV	R	CV	R	CV	
+ 17.5021	+43.1458	-7.8596	+ 80.8891	+ 37.6440	+ 3.0526	+103.7263	+ 66.9913	-13.4587	+ 8.9027	+24.0370	-13.1761	+ 37.4751	+ 10.6830	+12.5321	+ 49.6991	+ 36.5002	-5.0031			
- 11.1111	+ 6.9985	-7.8054	+ 22.2222	+ 7.6753	- 3.2659	+103.8461	+ 37.1350	- 8.9054	-24.1379	-17.5950	-19.3396	+182.6087	+ 78.5232	+ 9.9609	+ 54.6856	+ 22.5474	-5.8711			
+ 38.0952	+24.1960	-1.4035	- 23.3333	+ 4.1040	-20.1290	+ 67.7419	+ 56.9093	-17.7314	-13.4615	-12.3167	+19.3995	+ 30.1370	+ 5.8145	-11.6022	+19.8359	+ 15.7414	-6.2933			
+ 12.5000	+ 6.2313	+8.0000	+ 50.0000	+ 21.5363	- 7.8673	+ 41.667	+ 7.5005	- 4.0019	+28.5714	+21.3844	- 3.5156	+100.0000	+ 55.9485	- 6.0543	+46.5476	+ 22.5202	-2.6878			
+ 28.7377	+53.0348	-5.5941	- 5.1448	+ 1.5952	- 6.2048	+197.7401	+138.2918	-10.6102	-16.9556	- 2.9685	- 6.8752	+105.1404	+ 24.6559	+12.4609	+61.9036	+ 42.9218	-3.3647			
+857.7640	+110.5396	+4.2283													+857.7640	+110.5396	+4.2283			
+120.5882	+14.2720	+0.8048													+120.5882	+ 14.2720	+0.8048			
+207.5000	+54.8692	-4.1522													+207.5000	+ 54.8692	-4.1522			
+214.2857	+59.3894	-1.1992													+214.2857	+ 59.3894	-1.1992			
+400.1988	+96.0258	+7.6356													+400.1988	+ 96.0258	+7.6356			
+ 38.3113	- 4.8795	-4.6821	+191.0853	+ 52.0611	+12.6035	+235.8670	+126.1822	- 2.4220	+64.5522	+53.0760	- 4.8952	+134.7340	+ 28.3341	+ 2.8368	+132.9100	+ 50.9548	+0.6882			
+ 76.9231	+44.5470	-4.7382	+ 67.5000	+ 18.8397	+ 5.8968	+203.2258	+ 70.4227	- 5.8820	+62.8571	+38.9161	- 7.7598	+ 64.5161	+ 24.0526	- 1.8547	+ 95.0044	+ 39.3556	-2.8676			
+127.5862	+49.0900	+2.8323	+165.2174	+ 62.4461	+ 3.1696	+256.6667	+ 77.3063	-13.5202	+51.2821	- 3.9350	+11.7259	+194.2857	+ 89.6797	-12.7551	+159.0076	+ 54.9174	+3.6986			
+ 10.0000	-10.0803	+1.4600	+ 14.2857	- 10.0075	- 5.4549	+ 53.8462	- 0.1445	- 5.4685	+58.3333	+18.4923	- 0.1296	+ 60.0000	+ 10.0800	- 1.8414	+ 39.2930	+ 1.6680	-2.2869			

been low and negative for the rest of the characters. In P₂, all characters except leaf number exhibit positive shift. Moreover, leaf length and tiller length show high magnitude. In P₃, slight negative shift is found for all characters except tiller number which shows high positive deviation. Leaf length, leaf breadth and leaf number in P₄ exhibit low negative change, tiller length shows low positive shift whereas tiller number has exhibited high positive deviation. In contrast to other families, P₅ exhibits high negative shift for tiller number. Deviation for other characters is low. However, leaf number and leaf breadth have shifted negatively.

It is encouraging enough that in some cases, particularly for leaf length, leaf breadth and tiller number positive shift of population mean has been realised. It indicates bright changes of recovery of some positive variants for individual characters and also promise for isolating some beneficial variants with high yield when selected on the basis of leaf length, leaf breadth and tiller number. It, however, in any case does not annul the possibility and scope of selecting advantageous variants from the upper boundary of the wide range of variation exceeding the control realised in those families showing negative shift.

The dose-wise averages of deviation of range and coefficient of variation reveal successful induction of variation due to irradiation. In general, the extent is more in 6 kR dose followed by 9 kR. The 3 kR dose exhibits comparatively low induced variation.

The dose-wise averages of range reveal increase universally. So far as the range for individual families are concerned, increase

over control is evident in all cases except leaf breadth in P₁, tiller number and tiller length in P₂ and leaf breadth, tiller number and tiller length in P₄ from 3 kR dose.

On the basis of dose-wise coefficient of variation increase of variation, in general, is evident. The extent is also high in all cases except tiller number in 3 kR, leaf breadth in 6 kR and leaf number in 9 kR dose.

So far the individual families from 3 kR dose are concerned, increase of variation is high for leaf length, tiller number and tiller length in P₁, leaf length and leaf number in P₂, all characters except leaf number in P₃ and leaf breadth, leaf number and tiller length in P₅. In P₄, increase is high for leaf length and leaf number; leaf breadth, tiller number and tiller length exhibit slight decrease of variation. In all the remaining cases, slight increase has been found.

In regard to the families from 9 kR dose, high increase is observed for leaf breadth and tiller number in P₁, slight increase is shown by tiller length and slight decrease is exhibited by leaf length and leaf number. In P₂, leaf breadth shows slight increase, leaf number shows slight decrease and the other three characters exhibit high increase of variation. In P₃, high increase is manifested by all characters except leaf number which shows slight decrease. In P₄, tiller number shows slight decrease, leaf number shows slight increase and other three characters exhibit high increase. P₅ shows high increase in all characters except leaf number which exhibits slight increase.



FIG. 1 - INDUCED MORPHOLOGICAL VARIATION AND
A SELECTED CLUMP (INDICATED BY A SCALE)
IN A VM₂ FAMILY (A PORTION)



The data also reveal that the range exhibits high increase in all those cases where coefficient of variation has shown high increase barring that for leaf length in P_1 and P_4 from 3 kR dose. The induced morphological variations in the VM_2 families are also visually detectable (Figs. 1 and 2).

The high extent of induced variation, thus realised, may pave the way for both directed as well as disruptive selection.

Isolation of clumps

Considering the shift of value away from the mean of the respective control in the 11 VM_2 families, the clumps can be conveniently discriminated on the basis of individual and collective performance of the five agronomic characters. Table No. 8 shows the frequency of clumps with shifting value for each of the characters. As mentioned earlier, the difference in number of clumps in the different families is due to the availability of surviving 'bibits'. Survivability of 'bibits' derived from 6 kR has been highest facilitating the turn over of 169 clumps in a single family.

The clumps showing predominant negative shift of values for five agronomic characters are designated as subvitals. These clumps are visually distinguishable from the control clumps (Figs. 3 and 4). Peculiarly enough, the percentage of subvital clumps is lowest in 9 kR and highest in 3 kR, while the percentage of promising clumps is highest in 6 and 9 kR.

The possibility of recovery of around 14% of promising clumps appears to be quite high, notwithstanding the fact that selection pressure was applied simultaneously for leaf length, leaf breadth



FIGS. 3 & 4 - SUBVITAL CLUMPS



and tiller number. It will not be out of place to mention here that the realization of so many promising clumps has been possible through dechimerization in VM₂ generation. The clumps isolated in a family which has a little or no dechimeric effect might show only subtle difference amongst themselves.

Leaf weight has high positive correlation with oil yield from leaf due to its high indirect effect. Positive shift of leaf weight compared to mean of respective control has been observed in all the promising clumps of 3 kR and 6 kR treatments as well as in the P₂, P₄ and P₅ families of 9 kR treatment. The one promising clump in P₁ of 9 kR has not shown shift towards positive or negative direction, while one clump each out of the promising clumps in P₃ of the same treatment has shown no deviation and shift towards negative direction. The clumps with normal or negative value are rejected. The highest per cent of clumps selected finally on the basis of leaf weight is from P₂ family of 9 kR, followed by P₅ of 3 kR, P₁ of 6 kR and P₅, P₄ and P₃ of 9 kR in that decreasing order.

Table No. 9 shows that the shift of mean values of the families with selected clumps in positive direction is appreciable for almost all the characters. The mean value for leaf number has shifted negatively but the shift is not high from the control except in case of P₅ from 9 kR where the value has not changed at all. The gain in leaf weight over the respective control has also been impressive. In spite of improved agronomic characters in the selections from 3 kR and 6 kR treatments, the oil recovery from leaf was lower than the respective controls. As such these two selections of 7.55% and 14.20% clumps from 3 kR and 6 kR treatments respectively will not find immediate use as superior clones. However, the impressive

increase in leaf weight might compensate oil yield per clump. Further information on the performance of these selections, particularly for the oil yield per hectare would identify their superiority.

TABLE NO. 9

CHANGES OF MEAN VALUES OF SELECTED CLUMPS
(IN % OF RESPECTIVE CONTROLS)

SELECTED CLUMPS FROM	CHARACTERS							OIL RECOVERY FROM LEAF
	LEAF LENGTH	LEAF BREADTH	TILLER NUMBER	LEAF NUMBER	TILLER LENGTH	LEAF WEIGHT		
3 kR P ₅	+15.9189	+10.0977	+16.0221	-7.5862	+15.8719	+75.8255	- 4.6512(4)	
6 kR P ₁	+22.3767	+11.2606	+33.5010	-0.0632	+23.9558	+88.3456	-21.6000(12)	
9 kR P ₂	+24.5079	+13.6573	+26.8136	-4.1377	+20.2819	+80.0578	+ 2.9200(12)	
9 kR P ₃	+15.5523	+15.4867	+94.9904	-1.4085	+12.3495	+71.8776	+ 9.1767(2)	
9 kR P ₄	+ 3.9033	+ 5.0071	+13.6232	-0.4695	+ 5.1969	+62.5085	+16.0000(1)	
9 kR P ₆	+15.2975	+ 8.9551	+15.9420	0	+12.8892	+65.9818	0 (3)	

Figures in parenthesis are number of clumps tested for oil recovery from leaf.

The clumps of the families P₂, P₃ and P₄ of 9 kR have shown increase in oil recovery from leaf over 2% to 16%. Though oil recovery has remained unchanged compared to control, the increase in leaf weight in the clumps of the family P₅ of 9 kR treatment positively indicates an improvement. Further improvement for oil recovery through selection of clumps from the upper boundary is possible.

It is apparent that estimated oil yield in the selected clumps of the VM₂ families will be comparatively higher than the respective controls. It may be recalled here that there are reports delineating improvement of this species through chemical mutagenesis. The facts

emanating from such reports indicate stimulating effect of chemical mutagens. But the reports on the effect of ionizing radiation in this species have not indicated any improvement of agro-economic characters observed in VM₁ generation. Considering the fact that Java citronella is a vegetatively propagated plant, the treated generation actually constitutes a heterogeneous population which includes the spectrum of somatic mutations produced in the vegetative buds of the 'bibits'. The partitioning of this heterogeneity in the VM₂ generation has actually helped in the identification and isolation of both promising and deleterious somatic mutations.

Oil composition of selected clumps

It is clear from the previous discussion that clumps of the following families of different X-ray treatments are agronomically superior for higher oil yield. The families are - (a) P₅ of 3 kR treatment, which includes 4 clumps, (b) P₁ of 6 kR treatment, which includes 24 clumps, (c) P₂, P₃, P₄ and P₅ of 9 kR treatment including 19, 2, 1 and 3 clumps respectively (Table No. 8).

GLC analysis of oil (Table No. 10) shows the three important components of oil expressed in percentage. The average composition of citronellal has been slightly lower than the control in the two clumps of P₅ of 3 kR. Geraniol has also the same fate, while citronellol has shown a 26% gain over the control. The average performance of 9 clumps belonging to P₂ of 9 kR also reveals the same trend. The 6 clumps of P₁ of 6 kR appears to be comparatively more promising for about 6% and 27% gain in citronellal and citronellol content respectively though there has been a slight depression in geraniol content.

Considering the oil composition with respect to the three components in individual clumps, clump-6 of 6 kR may be regarded to

TABLE No.10

CHANGES OF OIL COMPOSITION IN THE SELECTED CLUMPS
(IN PERCENTAGES)

SELECTED CLUMPS AND CONTROLS	CITRONELLAL	CITRONELLOL	GERANIOL
0 KR (CONTROL)	27.32	7.52	30.87
3 KR P ₅ 1.	26.16 (- 4.25)	9.61 (+27.79)	27.31 (-11.53)
2.	25.75 (- 5.75)	9.41 (+25.13)	32.05 (+ 3.82)
FAMILY AVERAGE	25.96 (- 4.98)	9.51 (+26.46)	29.68 (- 3.85)

0 KR (CONTROL)	26.81	8.83	27.42
6 KR P ₁ 1.	29.83 (+11.26)	10.23 (+15.86)	30.41 (+10.90)
2.	31.30 (+16.75)	12.11 (+37.15)	28.32 (+ 3.28)
3.	31.51 (+17.53)	11.69(+ 32.39)	26.54 (- 3.21)
4.	28.49 (+ 6.27)	9.83 (+11.33)	30.83 (+12.44)
5.	29.21 (+ 8.95)	12.15 (+37.60)	29.63 (+ 8.06)
6.	19.87 (-25.89)	11.44 (+29.56)	14.81 (-45.99)
FAMILY AVERAGE	28.37 (+ 5.82)	11.24 (+27.29)	26.76 (- 2.41)

0 KR (CONTROL)	24.56	8.64	31.73
9 KR P ₂ 1.	24.99 (+ 1.75)	8.72 (+ 0.93)	27.29 (-13.99)
2.	24.51 (- 0.20)	10.89 (+26.04)	31.85 (+ 0.38)
3.	24.35 (- 0.86)	9.44 (+ 9.26)	28.30 (-10.81)
4.	25.69 (+ 4.60)	10.10 (+16.90)	29.65 (- 6.56)
5.	23.98 (- 2.36)	8.78 (+ 1.62)	29.71 (- 6.37)
6.	26.70 (+ 8.71)	10.67 (+23.50)	27.62 (-12.95)
7.	17.87 (-27.24)	15.92 (+84.26)	46.64 (+46.99)
8.	18.19 (-25.94)	9.88 (+14.35)	22.97 (-27.61)
9.	30.06 (+22.39)	12.37 (+43.17)	33.12 (+ 4.38)
FAMILY AVERAGE	24.04 (- 2.12)	10.75 (+24.42)	30.79 (- 2.96)

Figures in parenthesis indicate the deviations from the respective controls, expressed in percentage.

be a citronellol mutant with over 29% gain while the citronellal and geraniol content have been reduced to about 26% and 46% respectively of the control. In case of clump- 7 of 9 kR the total alcohol content (citronellol + geraniol) has increased spectacularly, while the aldehyde content has been reduced to 27% of the control.

The extent of deviations in the aldehyde and alcoholic components of oil clearly indicate that X-ray induced mutation has influenced the biosynthetic pathways of the different components though citronellal and geraniol show both increase and decrease from the control. The induced increase of citronellol under the circumstances probably indicates a possible relationship in the synthesis of the two alcohols. Clump-7 of 9 kR showing increase in content in both the alcohols with concomitant decrease in the aldehyde content might, in the similar way, indicate a competition in the biosynthetic pathways of the alcohols and the aldehyde. However, the trends of change in composition in other clumps may reveal that the relationship in biosynthesis of the three components may be more complex. Further work is necessary to evaluate the biochemical background of oil composition with a view to facilitating breeding to evolve quality genotypes. Maximum increase has been observed in case of citronellol. The percentages of deviations from the respective controls are always positive and ranging between 0.93% to as high as 84.26%. In case of citronellal and geraniol the deviations in content have been both positive and negative. Clump-7 of 9 kR has the highest citronellol and geraniol content and the lowest citronellal content. Clump-9 of the same treatment shows increase in content of citronellal, citronellol and geraniol. Though the per cent gain of geraniol in this clump is not as high as the

per cent gain obtained in Clump-4, 1 and 5 of 6 kR treatment, the concomitant gain in citronellal and citronellol content in the latter clumps are not as high as that in the former clump. As a matter of fact, these four clumps are superior in terms of oil quality with a new pattern for the three components because of the increase in citronellol content. In contrast, Clump-6 of 6 kR, Clump-3 and 8 of 9 kR show loss in citronellal and geraniol content and gain in citronellol content only. Though the composition of oil in these clumps is not desirable, their superior agronomic qualities do not rule out the scope of utilizing these clumps for future breeding work.

SUMMARY AND CONCLUSION

'Bibits' of Cymbopogon winterianus Jovitt Cv. Java-II have been X-rayed at 3, 6, 9, 12 and 15 kR. LD₁₀₀ and LD₅₀ have appeared in 12 kR and 3 kR treatments respectively. The effect of irradiation is manifested by increased variation of leaf length, leaf breadth, tiller number, leaf number and tiller length. Their mean values in VM₁ generation mostly exhibit negative shift from those of the controls.) Initial screening of clumps for hardiness has been done in the VM₂ families. The net outcome is five families each from 3 and 9 kR treatments, which have shown 50% survivability and the one family from 6 kR treatment which has led to only 10% survivability at VM₁ generation.

Mean values for the five agronomic characters exhibit positive or negative shift away from the control and the range and coefficient of variation has increased, in general in the VM₂ generation.) The clumps of the VM₂ families have been categorized into normal types as well as positive or negative variants from comparison with the respective controls for individual characters. On the basis of simultaneous consideration of higher mean values for leaf length, leaf breadth and tiller number for isolating promising clumps in the VM₂ generation, the percentages of success have been over 7% in 3 kR and 14% in 6 and 9 kR X-ray treatments. The elite clumps are finally isolated from the primary selection on the basis of leaf weight. Only three promising clumps have been rejected from 9 kR treatment for the leaf weight being comparable or less than the control. The isolates from each VM₂ family indicate consistent increase over the respective controls for leaf length, leaf breadth, tiller number, tiller length and leaf weight. There is possibility of further selection from the VM₂ generation on the basis of one or two of the three characters, viz., leaf length, leaf breadth and tiller number. The selected clumps do exhibit either

increase or decrease of oil recovery per cent from the leaf, and mostly a negative shift for leaf number.

(X-ray treatment has modified the oil composition of some of the selected clumps. Increase of citronellol and citronellal in comparison to the respective control is spectacular as evidenced by an increase of only 0.93% to as high as 84.26% over the control for citronellol and increase of 1.75% to as high as 23.39% over the control for citronellal.) Geraniol content also has exhibited an increase of 0.38% to 46.99% as compared to control. X-irradiation has led to amelioration of oil quality in terms of one, two or all the three components.

Systematic screening of the effect of irradiation on the axillary buds of 'bibits' has facilitated the recognition of the expanded variation. The upper and lower boundary of the metric values of leaf length, leaf breadth and tiller number have transgressed in relation to that of control. Estimates of variation support this diversity. The final consideration of leaf weight for the screening of clumps has exposed the possibility of further isolation for improvement of oil yield. Notwithstanding the decrease in oil recovery per cent, selection on the basis of leaf weight subsequent to the three basic criteria, viz., leaf length, leaf breadth and tiller number, has facilitated the identification of elite clumps. The expanded range of variation has helped in the realization of such clumps.

Finally, the GLC analysis of oil composition has also revealed recognizable modifications. Most of the selected elite clumps show high citronellol content. Some like the clump-6 of 6 kR treatment has enriched citronellol content. (The selected clumps can be suitably

classified for enrichment in their alcoholic components in the oil and may be earmarked for diverse utilization in the industries.) The increase in aldehyde content of oil in clump-3 of 6 kR treatment is also not insignificant. Increase of geraniol content and citronellol content by 46.99% and 84.26% respectively over the control in clump-7 of 9 kR is quite appreciable. Clump-1, 4, 5 of 6 kR (P₁) and clump-9 of kR (P₂) have exhibited appreciable increase of all the three components. Citronellal and geraniol rich citronella oil is favoured by the industry as a starting material. Induced somatic mutation has helped in the diversification of types on the basis of oil quality.

FUTURE SCOPE OF WORK

Irradiation of 'bibits' has produced wide range of variation, particularly in the three agronomic character viz., leaf length, leaf breadth and tiller number, which are found to be directly involved in the control of oil yield. Selection on the basis of these three characters along with leaf weight has yielded a number of promising clumps showing improvement in oil yielding ability and oil quality. The spectrum of somatic modifications induced indicates adequate scope of further selection of clumps on the basis of individual criterion. Systematic selection from the upper boundary of modifications may give rise to specific agronomic types suitable for diverse agro-ecological situations. Further work is, therefore, necessary to evaluate the efficiency of specific agro-types superior for any one or two of the basic selection characters.

Secondly, such diverse agro-types may be used as donors for breeding by conventional hybridization technique to be carried out in agro-climates where Java citronella is reported to flower and to set viable seeds regularly. The induced variations coupled with the residual heterozygosity of the genotype would provide a wide base of variation for selection.

Thirdly, the clumps have been screened for hardiness. Further work is necessary to evaluate the level of tolerance of the selected clumps under minimal cultivation. Testing of the clumps in the dry laterite zone and isolation, if possible, of a well-adopted clone for that zone would go a long way to establish this crop in the current fallow and marginal land.

Last, but not the least is the important spectrum of variation in oil composition shown by the selected clumps. This provides a new horizon for mutagenesis of Java citronella with a view to evolving quality genotypes.

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* Original not seen.

**SOIL CHARACTERISTICS OF THE
EXPERIMENTAL PLOT**

Soil texture	Sandy loam
Sand	62%
Silt	19%
Clay	19%
Nutritional status of soil	
Organic carbon	0.65%
Total nitrogen	0.06%
Available P	20 kg/ha
Available K	90 kg/ha
Soil reaction	pH 7.2 (neutral)

**CLIMATIC FACTORS DURING THE GROWING
PERIOD FOR EXPERIMENT - I**

MONTHS (1980)	AVERAGE TEMPERA- -TURE (°C)		TOTAL RAINFALL (Cm)	AVERAGE RELATIVE HUMIDITY (%)	
	MAXIMUM	MINIMUM		MAXIMUM	MINIMUM
March	34.44	19.69	2.41	81.39	48.45
April	40.05	25.87	1.27	70.15	41.75
May	37.45	25.68	14.45	79.70	51.38
June	34.25	26.25	24.16	87.43	72.97
July	33.57	26.43	21.36	88.11	75.77
August	33.75	26.39	38.33	88.82	71.57
September	34.00	26.95	9.30	94.65	69.66
October	33.44	23.05	5.23	84.40	62.20

ANNEXURE - II

CLIMATIC FACTORS DURING THE GROWING
PERIOD FOR EXPERIMENT - II

M O N T H S	AVERAGE TEMPERA- -TURE (°C)		TOTAL RAINFALL (Cm)	AVERAGE RELATIVE HUMIDITY (%)	
	MAXIMUM	MINIMUM		MAXIMUM	MINIMUM
June, 1981	37.35	27.33	2.18	86.97	58.68
July, "	33.68	25.13	21.91	88.03	69.87
August, "	31.00	25.92	12.69	88.74	69.71
September, "	34.40	24.35	10.55	89.90	66.43
October, "	31.74	23.08	0	89.29	53.71
November, "	32.88	15.65	0	87.33	54.13
December, "	26.19	11.31	8.59	79.61	52.77
January, 1982	28.56	11.79	0	88.16	53.20
February, "	30.09	11.42	15.82	86.18	51.32
March, "	34.76	20.58	3.22	89.16	54.68
April, "	36.43	23.53	6.19	86.83	41.70
May, "	39.54	25.70	2.74	77.41	44.75
June, "	37.93	25.86	9.60	82.29	57.74
July, "	31.17	27.41	10.28	88.35	59.93
August, "	32.40	26.19	14.84	90.14	71.77

ANNEXURE - III

OBSERVATIONS ON VM₂ FAMILIES AND THEIR RESPECTIVE CONTROLS

CHARACTERS	R			CV			SE			R			CV			SE			/n	
				3 KR P ₁						0 KR (CONTROL)										
Leaf length (cm)	60.50-74.33	9.1881	2.6589	64.701	64.23-76.00	6.4187	2.0187	70.220												
Leaf breadth (cm)	1.46- 1.70	6.2577	0.0456	1.630	1.63- 1.90	5.8484	0.0462	1.768												
Tiller number	39.00-68.00	21.2929	5.3515	56.200	49.00-70.00	17.1446	4.3703	57.000												
Leaf number	5.00-5.90	6.5481	0.1581	5.400	4.60-5.40	6.1640	0.1378	5.000												
Tiller length (cm)	79.08-93.46	7.1605	2.7701	86.506	86.74-97.91	4.6790	1.9174	91.632												
				3 KR P ₂						0 KR (CONTROL)										
Leaf length (cm)	49.31-88.78	19.6367	3.9071	62.920	53.04-74.86	14.2663	3.2736	64.9012												
Leaf breadth (cm)	1.42- 1.75	6.9260	0.0341	1.555	1.49- 1.76	6.4323	0.0366	1.6075												
Tiller number	52.00-75.00	12.4777	2.4424	61.900	64.00-94.00	11.9858	3.2842	77.5000												
Leaf number	4.40- 5.60	7.4650	0.1147	4.860	4.90- 5.70	6.1422	0.1146	5.2750												
Tiller length (cm)	71.06-95.94	9.5848	2.6520	87.498	83.12-109.36	9.4343	3.1116	93.2862												
				3 KR P ₃						0 KR (CONTROL)										
Leaf length (cm)	39.14-72.49	15.7568	3.2580	62.0311	63.29-79.66	9.4357	3.0246	71.6780												
Leaf breadth (cm)	1.23- 1.76	10.0701	0.0536	1.5978	1.63- 1.89	7.3432	0.0576	1.7540												
Tiller number	26.00-78.00	36.3992	5.7295	47.2222	42.00-73.00	23.1976	5.9547	57.4000												
Leaf number	4.20- 5.90	9.5927	0.1602	5.0111	4.70- 5.90	8.9234	0.2083	5.2200												
Tiller length (cm)	65.02-101.91	13.3665	3.7140	83.3578	86.85-99.24	5.6093	2.3393	93.2520												

Contd.....

ANNEXURE - III (Contd.)

OBSERVATIONS ON VM2 FAMILIES AND THEIR RESPECTIVE CONTROLS

CHARACTER	R		CV		SE		R		CV		SE	
	3 KR P4											
Leaf length (cm)	47.02-68.06	13.9487	3.6598	58.6700	54.65-73.97	11.2456	3.3684	70.9784				
Leaf breadth (cm)	1.26-1.48	5.7091	0.0349	1.3680	1.52-1.81	6.9281	0.0526	1.7485				
Tiller number	80.00-125.00	19.3254	8.9363	103.4000	62.00-114.00	22.0400	8.5357	95.1357				
Leaf number	4.60-5.50	7.3826	0.1631	4.9400	4.80-5.50	6.0820	0.1393	5.2593				
Tiller length (cm)	76.64-96.28	8.9955	3.5386	87.9680	82.22-105.87	9.2707	3.9161	98.3721				
	3 KR P5											
Leaf length (cm)	60.08-87.74	11.5282	1.7177	73.0180	53.24-73.36	10.4128	2.3887	64.8837				
Leaf breadth (cm)	1.28-1.93	9.4437	0.0325	1.6879	1.37-1.60	5.2399	0.0287	1.5350				
Tiller number	34.00-129.00	29.8160	4.8689	90.0000	62.00-135.00	28.1776	9.0159	90.8000				
Leaf number	4.20-6.40	9.8116	0.1023	5.1083	4.90-6.00	6.2915	0.1210	5.4376				
Tiller length (cm)	86.13-124.84	9.1753	1.9848	105.9733	83.70-102.57	7.3505	2.4522	94.2312				
	6 KR P1											
Leaf length (cm)	35.80-97.48	16.3105	0.8944	71.2859	67.70-74.14	7.7470	2.3696	68.3940				
Leaf breadth (cm)	1.29-2.04	9.5192	0.0120	1.6409	1.43-1.77	8.2303	0.0606	1.6278				
Tiller number	12.00-135.00	36.0376	1.8493	66.7101	58.00-98.00	23.2697	7.2428	69.6000				
Leaf number	4.20-6.40	8.4733	0.0336	5.1574	4.90-5.60	5.3161	0.1241	5.2200				
Tiller length (cm)	57.70-133.18	14.0182	1.1051	102.4842	86.47-101.56	7.1512	3.0450	95.2140				

Contd.....

ANNEXURE - IV

STATISTICAL PROCEDURES

ANALYSIS OF VARIANCE

Source	d.f.	S.S.	M.S.	F
Replications	4	Sr ₁₁	Mr ₁₁	
Cultivars	2	Sv ₁₁	Mv ₁₁	<u>Mv₁₁</u>
Error	8	Se ₁₁	Me ₁₁	Me ₁₁
Total	14	St ₁₁		

$$C.D._{0.05/0.01} = \sqrt{\frac{2 Me_{11}}{r (5)}} \times t_{0.05/0.01} (2.306/3.355)$$

EXPECTATIONS OF MEAN SQUARES AND MEAN CROSS PRODUCTS

Source	Analysis of variance		Analysis of Covariance	
	M.S.	Expectation of M.S.	M.C.P	Expectation of M.C.P.
Replication	Mr ₁₁	-	Mr ₁₂	-
Cultivar	Mv ₁₁	$\frac{2}{r} \sigma_{e_{11}}^2 + \sigma_{g_{11}}^2$	Mv ₁₂	$\frac{2}{r} \sigma_{e_{12}}^2 + \sigma_{g_{12}}^2$
Error	Me ₁₁	$\frac{2}{r} \sigma_{e_{11}}^2$	Me ₁₂	$\frac{2}{r} \sigma_{e_{12}}^2$

COMPONENTS OF VARIANCE AND COVARIANCE

$$\sigma_{g_{11}}^2 = (Mv_{11} - Me_{11})/r \quad \sigma_{p_{11}}^2 = \sigma_{g_{11}}^2 + \sigma_{e_{11}}^2$$

$$\sigma_{g_{12}}^2 = (Mv_{12} - Me_{12})/r \quad \sigma_{p_{12}}^2 = \sigma_{g_{12}}^2 + \sigma_{e_{12}}^2$$

$$\sigma_{e_{11}}^2 = \text{Environmental variance} \quad \sigma_{e_{12}}^2 = \text{Environmental covariance}$$

$$\sigma_{g_{11}}^2 = \text{Genotypic variance} \quad \sigma_{g_{12}}^2 = \text{Genotypic covariance}$$

$$V_{P_{11}}^2 = \text{Phenotypic variance} \quad V_{P_{12}}^2 = \text{Phenotypic covariance}$$

GENETIC PARAMETERS

$$H\% \text{ (Heritability in broad sense percentage)} = \frac{V_G^2}{V_P^2} \times 100$$

$$GCV \text{ (Genotypic coefficient of variation)} = \frac{V_{G_{11}}^2}{\bar{X}_1^2} \times 100$$

\bar{X}_1 = Mean of variable

$$GA \text{ (Genetic advance)} = i \times H\% \times V_{P_{11}}$$

i = Standardized selection differential, 2.06 for selection of 5% population

$$GA\% \text{ of Mean} = (GA/\bar{X}_1) \times 100$$

PHENOTYPIC, GENOTYPIC AND ENVIRONMENTAL CORRELATION

$$r_p \text{ (Phenotypic correlation)} = \frac{V_{P_{12}}}{\sqrt{V_{P_{11}}^2 \times V_{P_{22}}^2}}$$

$$r_g \text{ (Genotypic correlation)} = \frac{V_{G_{12}}}{\sqrt{V_{G_{11}}^2 \times V_{G_{22}}^2}}$$

$$r_e \text{ (Environmental correlation)} = \frac{V_{e_{12}}}{\sqrt{V_{e_{11}}^2 \times V_{e_{22}}^2}}$$

SIMULTANEOUS EQUATIONS TO DEDUCE DIRECT AND INDIRECT EFFECTS

$$r_{16} = P_{16} + r_{12} P_{26} + r_{13} P_{36} + r_{14} P_{46} + r_{15} P_{56}$$

$$r_{25} = r_{12} P_{16} + P_{26} + r_{23} P_{36} + r_{24} P_{46} + r_{25} P_{56}$$

$$r_{36} = r_{13} P_{16} + r_{23} P_{26} + P_{36} + r_{34} P_{46} + r_{35} P_{56}$$

$$r_{46} = r_{14} P_{16} + r_{24} P_{26} + r_{34} P_{36} + P_{46} + r_{45} P_{56}$$

$$r_{56} = r_{15} P_{16} + r_{25} P_{26} + r_{35} P_{36} + r_{45} P_{46} + P_{56}$$

r -values are phenotypic correlation values for each pair of variables, P -values are direct path values of the 5 independent variables on the dependent variable.

$$PR_6 \text{ (residual effect)} = \sqrt{1 - (P_{16} r_{16}) - (P_{26} r_{26}) - (P_{36} r_{36}) - (P_{46} r_{46}) - (P_{56} r_{56})}$$

ANNEXURE - V

RESULTS OF ANALYSIS OF VARIANCE FOR
INTERVARIETAL VARIATION

C H A R A C T E R	M E A N S U M O F S Q U A R E S		
	Replications	Cultivars	Error
Oil yield from herb	6.6204	49.4668	4.5705
Oil yield from leaf	5.2301	31.1176	2.9204
Oil yield from stem	0.1392	2.8075	0.2088
Oil recovery from herb	0.0005	0.0241	0.0019
Oil recovery from leaf	0.0042	0.0733	0.0057
Oil recovery from stem	0.0008	0.0014	0.0005
Herb weight	260923.7	799575.0	134727.9
Leaf weight	91675.95	197487.8	28243.97
Stem weight	57907.95	379279.7	44953.97
Tikler number	1233.640	4972.872	529.1952
Leaf number	0.0557	0.6194	0.1537
Tiller length	72.2656	995.7499	21.0547
Leaf length	6.6563	725.2186	24.8125
Leaf breadth	0.0175	1.1618	0.0037
Stem length	9.3896	0.5762	2.2492