genes due to *dpp* loss-of-function mutation. This aspect is being examined further.

As has been reported earlier[26], most of the B^+; dpp^{d6}/dpp^{d12} males were completely devoid of external genitalia while a few had abnormal external genitalia. As in legs and antenna, the *Bar* mutation had an enhancing effect on male genitalia also since the external genitalia were absent in all *B*; dpp^{d6}/dpp^{d12} male flies (not shown). Interestingly, the external genitalia were not much affected in female flies of any of the genotypes. The enhancing effect of *B* mutation on male, but not female, external genitalia in *dpp* mutant background also warrants further study.

The classical view has been that the *Bar* locus has a function only in eye differentiation in view of its phenotypic effect being restricted to differentiation of ommatidia in eyes. Recently, this gene was shown to also function in the differentiation of the notal region of wing of *Drosophila*[8]. We have now shown that the *Bar* locus has roles in differentiation of legs and antennae (and possibly also the external male genitalia) as well. Thus it appears that this complex locus of homeo-box containing genes plays a much wider role in differentiation of different structures in *Drosophila*.

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Isolation and characterization of PR1 homolog from the genomic DNA of sandalwood (*Santalum album* L.)

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Genomic library was constructed using nuclear DNA prepared from tender leaves of sandalwood. Subsequently, screening with heterologous probes we could isolate the PR1 genomic homolog. Restriction mapping and hybridization experiments were carried out to obtain the coding region for *PR1* gene. A 750 bp *Eco*RI fragment thus obtained was subcloned to yield pSaPR1, which was compared with the related sequences. Southern hybridization with genomic DNA digests was carried out to check its genomic organization. The induction of this gene was observed in the somatic embryos treated with salicylic acid, thereby implying its possible involvement during systemic acquired resistance.

SELF defense in plants stems from the necessity of their survival against various pathogens. As observed, plants are being challenged constantly by various pathogens but disease is not always the inevitable outcome. Depending on the pathogen, plants exhibit different types of defense responses which can be classified into three classes according to their spatial and temporal occurrences[1]. The first class comprises immediate, early responses that

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involve changes in ion fluxes across the plasma membrane, synthesis of active oxygen species (oxidative burst)[2–4] and the hypersensitive reaction (HR). HR is characterized by a local necrotic lesion that effectively traps the pathogen to the site of infection and prevents its spread throughout the rest of the plant[4]. The second line of defense, thought to restrict the growth and development of pathogen, is activated at the site of infection. This response involves the *de novo* synthesis of several proteins including enzymes involved in phenylpropanoid metabolism, and the biosynthesis of phytoalexins and pathogenesis-related (PR) proteins. The third line of defense that can occur in many plant–pathogen interactions is triggered on in the non-infected parts of the plant, which is known as systemic acquired resistance (SAR). SAR is characterized by the protection of uninfected parts of a plant against a second infection by the same or even unrelated pathogen[5,6]. SAR implies the existence of a signal molecule produced in the infected tissue, that moves throughout the plant to activate resistance[7]. Salicylic acid (SA) has been proposed to have a central role as a signaling molecule leading to SAR as its concentration rises dramatically after pathogen infection[8–13]. Furthermore, exogenously applied SA leads to typical SAR responses such as increased resistance to viral infection[5,14,15]. Recent evidence suggests that SA may not be the primary long-distance SAR inducing signal and that the production of this systemic signal is not dependent on SA accumulation. However, SA is required in uninfected tissues for transduction of the translocated signal into gene expression and resistance[16].

SAR is associated with the systemic *de novo* synthesis of a large number of PR proteins. Their time of appearance and the known function of at least some of the PR proteins suggest their involvement in SAR. Some members of the PR family, chitinases and β-1,3-glucanases, inhibit fungal growth. Moreover, β-1,3-glucanases may release defense-activating elicitors. Direct evidence of the potential role of PR genes in plant defense has been obtained by the experiments which demonstrated that overexpressing PR genes can lead to enhanced resistance to certain pathogens[17,18]. Since PR genes are induced in parallel with the appearance of SAR, they are useful targets to develop protection strategies in plants. Moreover, in the systems where genetically defined resistance is not described or prior knowledge of pathogen avirulence gene is not available, development of disease resistance may be achieved by manipulating these sets of genes.

As part of a tree improvement programme we are trying to study the defense response in an economically important tropical timber tree sandalwood (*Santalum album* L.), which may be used later to develop disease-resistant plants. Towards this end, we have attempted to clone and characterize some of the PR genes from sandalwood. Here we report cloning and characterization of PR1 homolog from sandalwood genomic library. We could demonstrate the induction of this gene in the somatic embryos when treated with salicylic acid, thereby implying its possible induction during SAR.

Somatic embryos used for this study were obtained by direct somatic embryogenesis (G. Lakshmi Sita *et al.* unpublished work) from internodal segments of young shoots. Briefly, explants were inoculated in MS medium[19] supplemented with thidiazuron (TDZ) and 6-benzylaminopurine (BAP) for direct somatic embryogenesis. Globular embryos thus obtained were transferred to MS medium supplemented with gibberellic acid (GA). Three- to 4-week-old somatic embryos were used for induction with SA and other downstream applications.

High molecular weight DNA was prepared from the nuclei isolated from sandalwood leaves. Briefly, sandalwood leaves were frozen and pulverized in liquid nitrogen which was then suspended in 5 volumes of cold nuclei isolation buffer (NIB) (15% sucrose, 50 mM Tris pH 8.0, 50 mM EDTA, 5 mM $MgCl_2$, 5 mM mercaptoethanol, 150 mM NaCl). Once thoroughly mixed, the homogenate was allowed to pass through three layers of cheese cloth and then centrifuged at 1500 *g* for 10 min at 4°C. The precipitate thus obtained was resuspended in the same buffer containing 0.1% Triton X-100. After an incubation in ice for 10 min, a nuclear pellet was obtained by centrifugation at 100 *g* for 10 min at 4°C. This nuclear pellet was used for isolation of high molecular weight DNA by the standard procedure[20].

DNA partially digested with *Sau*3AI was partially filled in and subsequently cloned in the λGEM-11 half-filled arms as described in Promega Protocols. The genomic library containing 105 recombinants was screened with *Arabidopsis* PR1, PR2 and PR5 cDNA probes (kindly provided by Dr John Ryals, Navartis, USA). Out of 19 positive clones obtained after multiple rounds of screening, one that was positive for PR1 was taken up for further characterization. This genomic clone having the insert of size approximately 18 kb was subjected to restriction mapping followed by Southern hybridization to obtain the coding region for PR1. A 750 bp *Eco*RI fragment thus obtained was subcloned in pBluescript to yield pSaPR1. Sequencing was carried out using Sequenase Kit (ver 2.0) (USB Biochemicals, USA) following the manufacturer's instruction. The nucleotide sequence and the deduced amino acid sequence from the same is represented in Figure 1.

Genomic DNA was restricted with *Hin*dIII, *Eco*RI, *Sac*I and *Xho*I which do not have an internal site within SaPR1 and fractionated in 0.8% agarose gels. DNA was transferred to nylon membrane (Hybond N$^+$, Amersham Inc.) using TE-80 vacuum transfer system (Hoefer Scientific Instruments, USA). Blots were hybridized with SaPR1 probe. Probes were labelled with [α-^{32}P]dATP using Amersham's megaprime labelling kit. Hybridizations were carried out for 24 h at 42°C in 50%

formamide, 5 × Denhardt's solutions, 6 × SSC, 1% SDS, 100 g ml denatured Salmon sperm DNA and blots were washed finally at 0.1 × SSC, 0.1% SDS for 30 min at 65°C.

Total RNA was isolated from sandalwood somatic embryos (treated with SA or mock treated with water) by the GITC-acid phenol extraction method as described by Chomczynski and Sacchi[21]. 10 µg of total RNA was denatured in formamide, separated by electrophoresis through formaldehyde agarose gels and blotted to Hybond N^+ filters[22]. Blots were hybridized with SaPR1 probe. Probes were labelled with [α-^{32}P]dATP using Amersham's megaprime labelling kit. Hybridizations were carried out in the same conditions as described earlier. Filters were washed finally at 0.5 × SSC, 0.1% SDS for 15 min at 50°C. All RNA gels were routinely visualized with ethidium bromide staining and equal loading was confirmed by reprobing the same blot with RNA gene probe.

The sequence of the predicted amino acids was aligned with related sequences obtained from SWISS-PROT data base. When compared with the known PR1 sequences it reveals 37–49% homology in the coding region (Table 1).

gaattcatgccatgcacttccctttactccaattggctttctctaataggggaaaagccc
tccatttccgtcaaatgacccggcctcccctggctcttccaccgtccgggcttccttcc
tccctatgctatgctcccccggcgcaggcctaccacgcttcgcgcctgcggcgttttcgc
cagacggtctttgccagtagtgccatcctccccgctggctgtatgggcgggttttccctt
M G G F S L
gcttcagcgttcttgttaagcctatggataactggaacaattgttagcagtagagctcaa
A S A F L L S L W I T G T I V S S R A Q
aatagcgcacaagattattcaacggttcccaacgtgagggcagtgggtgtagagataacc
N S A Q D Y S T V P N V R A V G V E I T
ccgtgggatgagcagcgccttgctgcatccgctcggcaacgcgcatcagatttaaaaaca
P W D E Q R L A A S A R Q R A S D L K T
cggtgccggctcgtacactctcattcgccttacggggaaaacttagccataactagcggt
R C R L V H S H S P Y G E N L A I T S G
cactttactacctttctcgccttcctccccatgtgggttgtcgagaagtttaactacgcc
H F T T F L A F L P M W V V E K F N Y A
gccacgctttgcgtttgccaccaagccgccttacggggttgcgctagaaagtcaaatggg
A T L C V C H Q A A L R G C A R K S N G
ggaaccaagcaggccagcagcaatggaccgactggagtggttccgtactagcgactcggc
G T K Q A S S N G P T G V V P Y *

gtcagcggcttcgtgccgcactggagtgatccaatatgtggttccgcacgttccaccacgaattc

Figure 1. Nucleotide and deduced amino acid sequences of the SaPR1. The genomic fragment contains 725 nucleotides. The stop codon is marked with an asterisk.

Table 1. Percentage of identity of aligned amino acid sequences of PR1

	Tobacco PR1c	Tobacco PR1a	Tobacco PR1b	*Arabidopsis* PR1	Maize PR1	
SaPR1	47.9	47.1	46.3	45.4	36.7	Per cent identity
	121	121	121	119	109	amino acid overlap

Organization of SaPR1 sequences in the genome was made by genomic blotting experiments. Genomic DNA was cut with *Eco*RI, *Hin*dIII, *Xho*I, *Sac*I restriction enzymes which do not cut within the 750 bp EcoRI fragment used as probe. As shown in Figure 2, the probe hybridizes predominantly to single fragments at high stringency in all four digests. In addition to this, other weak bands could be detected in some of the digests. To determine whether these fragments were due to partial digestion of DNA, the same digests of DNA were probed with other control DNA and the banding patterns indicated complete digestion (data not shown). Together, these results suggest that SaPR1 may react with other members of this gene family.

Expression of PR1 was checked in the somatic embryos mock treated with water and upon treatment with SA. Figure 3 shows that there is an induction of PR1, when the embryos were treated with SA. The probe hybridizes specifically to a single mRNA species of size 0.8 kb. Longer exposure of the blot results in the appearance of faint signals in the uninduced lanes (data not shown). This corroborates well with the other reports of PR1 induction in various plants, thus suggesting the possible involvement of SaPR1 during SAR.

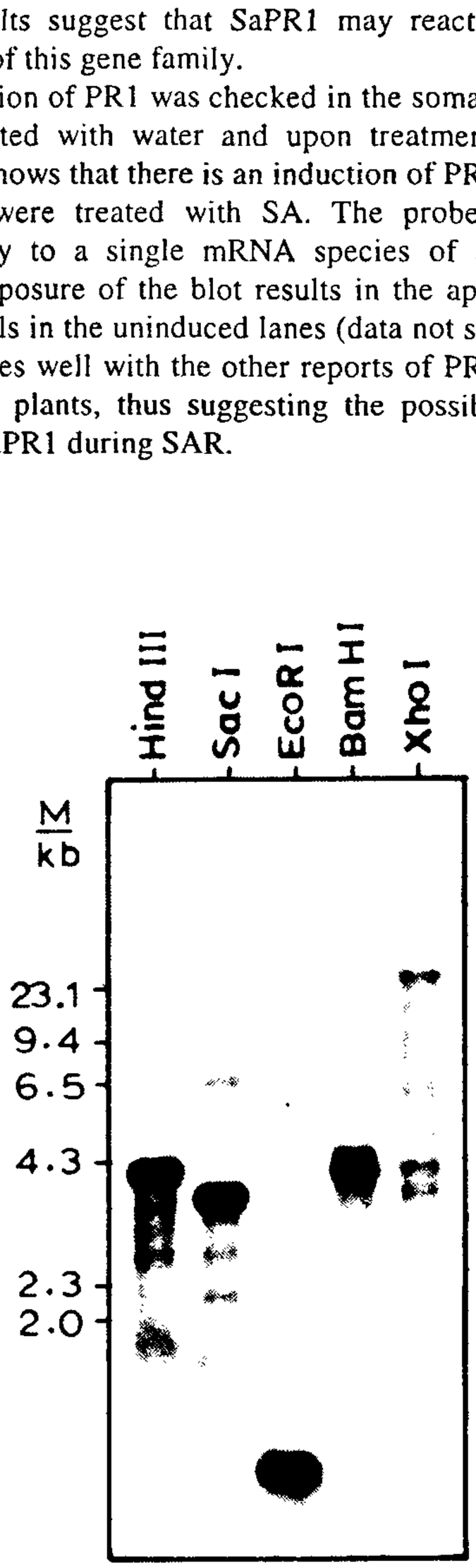

Figure 2. Southern hybridization analysis. Genomic DNA (10 µg per lane) was digested with the indicated restriction enzymes. The migration of the molecular weight standards are indicated.

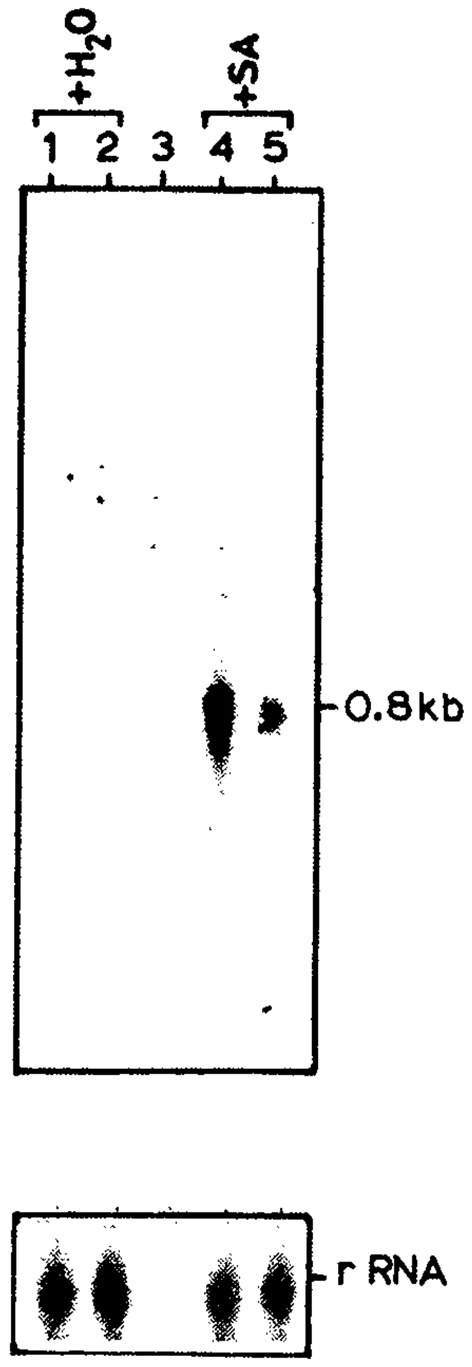

Figure 3. Expression of *SaPR1* transcript in SA-treated somatic embryos. 10 µg of total RNA on each lane from uninduced and induced embryos was loaded as indicated. Upper panel shows the expression of *SaPR1* transcript in the induced embryos. The same blot was reprobed with rRNA gene as shown in the bottom panel. Lanes 1 and 2, RNA from water-treated embryos in different periods; Lane 3, RNA from control (no treatment) embryos; Lanes 4 and 5, RNA from SA-treated embryos in different periods.

In summary, a genomic library was constructed from an economically important tropical timber tree, sandalwood and screened with *Arabidopsis* PR1, PR2 and PR5 cDNA probes. PR1 reading frame was identified from a genomic fragment, which codes for a putative protein of 143 amino acids and an estimated molecular mass of 15 kDa. The *SAR* gene shares 43–47% amino acid sequence identity with various *PR1* genes. Southern hybridization was carried out using SaPR1 probe to show its possible homology with other members of the gene family. Thus we have successfully demonstrated that this gene is induced by SA, and hence probably during SAR, by northern blot analysis.

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Inheritance of anther culture response in rice

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F_1 hybrids of a set of crosses involving the highly-responding *Japonica* cultivar, Taipei 309 and the three low-responding *Indica* cultivars, IR72, IR64, and IR54, as well as the parents were studied for their response to anther culture in terms of callus induction and plant regeneration frequency. Diallel analysis revealed the high-response in Taipei 309 to be governed predominantly by recessive alleles as against the low-responding *Indica* cultivars which were largely due to dominant alleles. Although it is difficult to suggest precisely the number of genes that control anther culture response, the study amply demonstrated that these traits followed quantitative mode of inheritance. Furthermore, our studies on the estimates of genetic components of variation for callus induction revealed that additive variance largely govern the response indices, suggesting thereby that transfer of the high callusing ability of Taipei 309 to low-responding genotypes by simple selection techniques should be feasible.

RESEARCH on anther culture in rice has generated large volume of information and material of basic and applied value over the past three decades. Nevertheless, exploitation of this technique in crop breeding and genetics research is hampered due to very low regeneration frequency of anthers of rice in general, and *Indica* cultivars in particular. Factors that restrict the use of anther culture to certain species/cultivars have been extensively reviewed recently[1]. Among them, influence of genotype is considered to be the most important in rice, as evidenced from inter-varietal and intra-varietal differences in anther culture response[1–3]. Research efforts on the enhancement of response to anther culture have, however, been confined mostly to manipulation/refinement of callus-induction and plant-regeneration protocols.

Earlier efforts to understand the genetics of anther culture response in some of the cereals including barley, wheat, maize, and rice revealed no consistency as the reported mode of inheritance varied from simple Mendelian to complex quantitative mode of inheritance[4–8]. Furthermore, the reports on number of genes or gene blocks that control anther culture response in rice is quite conflicting. Whereas Miah *et al.*[7] reported callus induction from anthers to be controlled by a single block of genes, Takeuchi *et al.*[9] opined involvement of two dominant genes governing callus induction, and plant regeneration. A recent report[10] on the molecular basis of anther culture response reveals the possible involvement of five quantitative trait loci (QTL) in the control of callus induction in the *Japonica* variety JX17, suggesting that the trait could be genetically as complex as the well-established quantitative traits, like yield. Since adequate information is lacking on the genetic control of callus induction and green plant regeneration from anthers, and also since inconsistency still persists on the nature and mode of gene action governing them, the present study was therefore, undertaken to elucidate further the genetic basis of anther culture response.

For the experimental studies four parents consisting of three low-responding *Indica* cultivars, viz. IR72, IR64, and IR54; and a high-responding *Japonica* cultivar, Taipei 309 were crossed in all possible combinations, including reciprocals. The material was evaluated in a randomized complete-block design with three replications. Anthers from these plants were plated for callus induction following the procedure described by Balachandran *et al.*[11]. The N6 basic medium[12] supplemented with 5% maltose, 2.0 mg/l 2,4–D, and 0.5 mg/l kinetin for callus induction; and MS medium[13] comprising 3% sucrose, 2.5 mg/l BAP, 0.5 mg/l kinetin, and 1.0 mg/l NAA for regeneration were used. Diallel analysis was carried out following Hayman's model[14,15] and heritability was estimated using the method of Mather and Jinks[16].

Analysis of variance showed highly significant differences among the parents and hybrids for callus induction (Table 1). The mean response to callus induction was higher in Taipei 309 (25.8%) than in the *Indica* cultivars, viz. IR72, IR54, and IR64 (Table 2). Likewise, F_1 hybrids with Taipei 309 as one of the parents also showed moderately high response compared to *Indica* × *Indica* hybrids. Similarly, in respect of plant regeneration, the F_1 hybrids having Taipei 309 as one of the parents invariably regenerated green plants at higher frequency than the *Indica* × *Indica* hybrids. The parents that yielded more calli exhibited a tendency to regenerate plants proportionately at higher frequency. Further, no significant reciprocal differences were observed among the hybrids for both the traits (Table 2).

The estimates of genetic components of variation for callus induction revealed the magnitude of D (additive genetic variance) and H_1 (dominance variance) to be

Table 1. Combined ANOVA for callus induction in 4 × 4 diallel cross

Source	DF	SS	MSS	F-value
Parents	3	1037.50	345.83	94.29**
F_1 hybrids	5	330.37	66.07	18.02**
Parent × F_1 hybrids	1	9.89	9.89	2.70
Replication	2	27.52	13.76	3.75*
Error	18	66.02	3.67	1.00

*, **Significant at 5% and 1% level of probability respectively.

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positively significant. As the D value was much higher than H_1, we can conclude that the additive component is predominant in the expression of the trait. This is in agreement with all the earlier reports that have associated callus response to both the additive component as well as the dominance component[7,8,17,18]. The observation that recessive and dominant alleles are possibly associated with both positive and negative effects, respectively, on callus induction (Figure 1), is in conformity with the findings of Imuta *et al.*[19]. Regression coefficient ($b = 1.24 \pm 0.27$), significantly different from zero but not from unity, is suggestive of the absence of epistasis as well as correlated gene distribution in the parents. Estimate of the mean degree of dominance over the loci ($H_1/D^{1/2}$) was less than unity (0.76), suggesting partial dominance. Relatively low (0.20) estimates of the ratio of negative to positive alleles, $H_2/4H_1$, indicates unequal distribution of alleles at the loci, exhibiting dominance in the parental genotypes. Although the positive sign of F suggests involvement of largely dominant alleles rather than recessive ones in controlling callus induction in the parents, it is implicit from high proportion of K_d/K_r (2.08) that the alleles responsible for high-callus induction were recessive. The estimated value of narrow sense heritability was high (70%), confirming further the major contribution of additive component to callus induction (Table 3).

The nature of gene action revealed that high-callusing ability is governed by recessive alleles, and that it would respond to selection as it is being governed predominantly by easily fixable additive component. By virtue of its being controlled by additive component, transfer of the high-callusing ability of Taipei 309 to low-responding *Indica* genotypes by adopting simple selection techniques should be feasible, as has been demonstrated in barley[20].

In one of the crosses, viz. Taipei 309 × IR64, anther culture response of 50 randomly selected F_2 plants was compared with the F_1 plants, and a continuous variation was observed, showing skewness either on the low or high-response direction in the F_1 itself. The frequency distribution of plants varying in callus-induction response was near normal in the F_2, encompassing the range of both high-response parents as well as the low-response ones, and the skewness showed a tendency towards low-response parents (data not shown). Because of the failure to fit the data on the different response groups into possible oligogenic Mendelian ratios, and also because the distribution pattern showed higher number of plants in the F_1 and F_2 generations falling in the low-response

Table 2. Relative anther culture response and reciprocal effects in intersubspecific hybrids*

Parents (P)/cross	Mean (%)	
	Callus induction	Green plant regeneration
Taipei 309 (P)	25.80a	20.17a
IR72 (P)	5.97de	10.47b
IR64 (P)	2.77ef	2.50c
IR54 (P)	4.73ef	6.30bc
Taipei 309 × IR72	13.53b	4.33bc
IR72 × Taipei 309	12.50bc	5.27bc
Taipei 309 × IR64	9.27cd	6.73bc
IR64 × Taipei 309	10.90bc	4.67bc
Taipei 309 × IR54	10.57bc	4.70bc
IR54 × Taipei 309	10.63bc	3.77bc
IR72 × IR64	1.70f	0.0c
IR64 × IR72	2.70ef	2.10c
IR72 × IR54	4.33ef	0.0c
IR54 × IR72	2.67ef	0.0c
IR64 × IR54	1.93f	0.0c
IR54 × IR64	1.63f	0.0c
C.D. (5%)	4.55	7.78

*Mean of three replications; Means followed by the same alphabet are not significantly different at 5% level by DMRT.

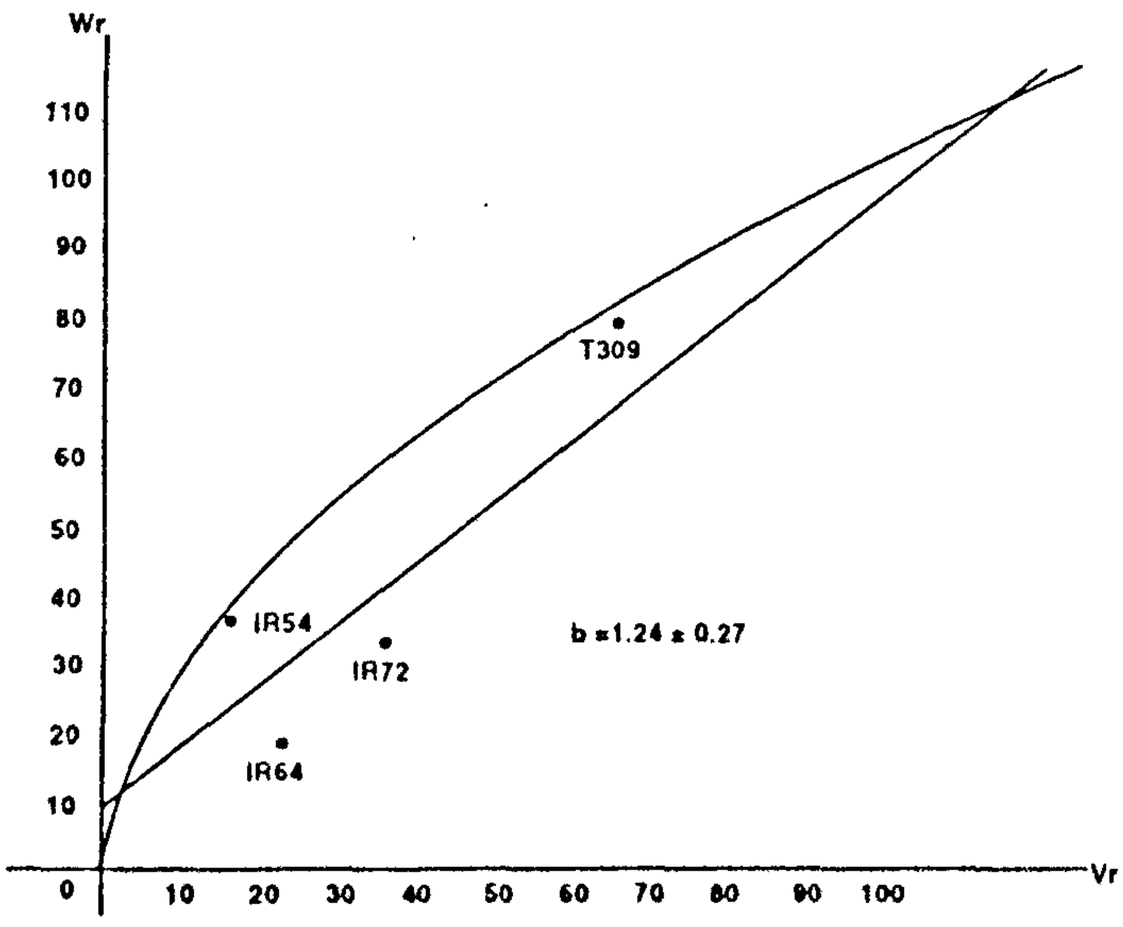

Figure 1. Graph of (*Wr*, *Vr*) for callus induction in 4 × 4 diallel cross.

Table 3. Estimation of genetic and environmental components of variation for callus induction in 4 × 4 diallel cross

Component	Estimated value ± SE
D (Additive)	113.91 ± 10.31**
H_1 (Dominance)	66.42 ± 29.96*
H_2 (Dominance)	52.52 ± 27.65
h^2 (Dominance)	2.06 ± 18.76
F (Additive, dominance, gene asymmetry)	61.09 ± 26.48*
E (Environmental)	4.61 ± 1.37
Proportional values	
$[H_1/D^{1/2}]$	0.76
$[H_2/4H_1]$	0.20
$[K_d/K_r]$	2.08
Heritability (ns)	0.70

*,**Significant at 5% and 1% probability levels respectively.

range, the data is not only indicative of the typically quantitative nature of the trait but also confirms the recessive nature of genes that control anther culture traits. Thus, the findings of present study as well as reports from both conventional and molecular studies stress further, the involvement of complex quantitative mode of inheritance governing anther culture traits. But, in-depth study using molecular tools is warranted to understand still more precisely the number of genes involved and their nature of control of anther culture response.

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Palaeomagnetism of Deccan Traps from the Killari borehole flows

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Borehole drilling in Deccan Traps at the Killari village, Latur District, Maharashtra, revealed nine Deccan Trap flows with a thickness of 338 m resting over a Precambrian basement. A palaeomagnetic study of drill core samples from these flows using laboratory AF and thermal demagnetization methods revealed downward pointing magnetic inclinations with a mean value of + 56.3°. This suggests that these flows were erupted during a reverse polarity period of the geomagnetic field. The mean magnetic inclination of these flows is equal to the reported inclination of the eastern and south-eastern part of the Deccan Traps. From this correlation it appears that most of the Deccan Traps in the region were poured out at the same time supporting the suggestion that the Deccan flood basalts were erupted in a very short period of time.

THE Deccan Traps occupying an area of half a million square kilometers in the western part of peninsular India constitute a single geologic unit, the palaeomagnetism of which was well studied. Several geochronologic results were also reported on these rocks giving the most probable age for the eruption of these flood basalts. Vandamme *et al.*[1] summarized the palaeomagnetic and geochronologic data of several flows of the trap country and made the following important inferences. Only two field reversals of the geomagnetic field occurred with a chron sequence of N–R–N. About 80% of the flows were poured out during the reverse chron which was correlated with Chron 29 R. Most of the Deccan Traps were poured out in a very short period of time (less than one million years). The Ar/Ar dating of the Deccan Traps assigns an absolute age of 65.5 ± 2.5 Ma for the volcanic eruption.

The large magnitude earthquakes ($M > 6.0$) at Koyna (1967) and Latur (1993) point towards a relatively enhanced level of seismotectonic activity of the Deccan Volcanic Province (DVP) in the Indian shield which is otherwise considered to be a stable region. With a view to understanding the configuration and structure of the basement, several boreholes were drilled jointly by NGRI and AMD in the Deccan Traps near Killari village (18°03′07″N, 76°33′20″E), the site of the 30 September 1993 earthquake. We have carried out palaeomagnetic investigation of the Deccan Trap flows encountered in the borehole about 2 km north-west of the Killari village to find out only the magnetic polarity. This is a unique opportunity to study a vertical sequence in the region.

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