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Short Communication

Peroxidase polymorphism for fingerprinting of black pepper (*Piper nigrum* L.) varieties and micropropagated clones

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The comparison of field performance of the micropropagated clones with those of conventionally propagated clones by band-to-band comparison of isozyme profiles is useful in assessing the real applicability of *in vitro* propagation [1, 2, 3]. In the present investigation, the clonal fidelity and varietal diversity was assessed in black pepper (*Piper nigrum* L.) using peroxidase polymorphism exhibited by 20 conventional clones (CC-raised by rooted cuttings) and 38 tissue culture derived clones (TCC) of the varieties Panniyur 1 (P_1), Panniyur 2 (P_2), Panniyur 4 (P_4) and Subhakara (Su). Enzyme was extracted from 4g mature leaf pieces (5th to 7th dark green leaves from tip) using 5ml Tris buffer (0.15M, pH 7.5-7.6) containing sodium metabisulfite and L-ascorbic acid (0.1M). The supernatant obtained by centrifugation (15000 rpm for 15 minutes at 4°C) was stored in aliquots at -20°C up to four days without any significant decrease in activity. Best resolution was obtained when electrophoresis was carried out using polyacrylamide gel (8.5%) prepared with Tris buffer (0.0375 M for resolving gel and 0.0125M for stacking gel, pH 8.9) and Tris-Glycine as tank buffer (0.0125 M Tris-0.096 M Glycine, pH 8.3). The staining was done by the standard procedure [4]. *P. colubrinum* sample was loaded in the last well of every gel in order to act as an internal marker for comparison. The relative mobility (Rm) value of individual isozyme band was calculated by measuring the distance travelled by the band relative to that of the marker band.

The electrophoretic pattern showed that there exist three major zones of activity and within each zone, there were several bands. Occurrence of several zones of activity for peroxidase was reported earlier [5, 6]. The nomenclature of the zones and bands were done according to the rate of anodal migration (Table 1). Pooling the number of bands from all the 58 vines, there were a total of 16 bands distributed in the three zones. It is characteristic of peroxidase that its isozyme pattern is complicated and difficult to interpret [3, 5, 6].

Table 1. Number, distribution and relative mobility of isoperoxidases in black pepper

Zone of activity (according to rate of migration)	Bands per zone	Relative mobility of bands	
		Band (according to rate of migration)	Rm
PRX-I (fastest)	7	PRX-I (1)	1.12
		PRX-I (2)	1.10
		PRX-I (3)	1.05
		PRX-I (4)	1.03
		PRX-I (5)	1.00
		PRX-I (6)	0.96
		PRX-I (7)	0.94
PRX-II	8	PRX-II (1)	0.92
		PRX-II (2)	0.88
		PRX-II (3)	0.84
		PRX-II (4)	0.82
		PRX-II (5)	0.80
		PRX-II (6)	0.76
		PRX-II (7)	0.72
		PRX-II (8)	0.68
PRX-III (slowest)	1	PRX-III (1)	0.45
Total	16		

Within conventionally propagated vines of each variety no polymorphism was detected showing the true-to-type nature. But within micropropagated clones one or two TC plants in each variety exhibited some polymorphism with respect to the presence or absence of some bands. Among the TC plants of P_1, the variant TC P_1-1 had two additional bands, one each in zones PRX-I and PRX-II, both slow moving in the respective zones. In variety P_2, the bands appeared continuously without any demarcation of different zones, as the fast migrating zone was not completely moved towards the anode. There were a total of 13 bands in all the TCC as well as clonal samples except TC P_2-7. Polymorphism in TC P_2-7 was exhibited as absence of six bands out of the thirteen and comparatively low intensity for the remaining seven bands. PRX-I (2) and PRX-I (3) were characteristic of this variety and were present in all including the variant. In P_4, there were nine monomorphic

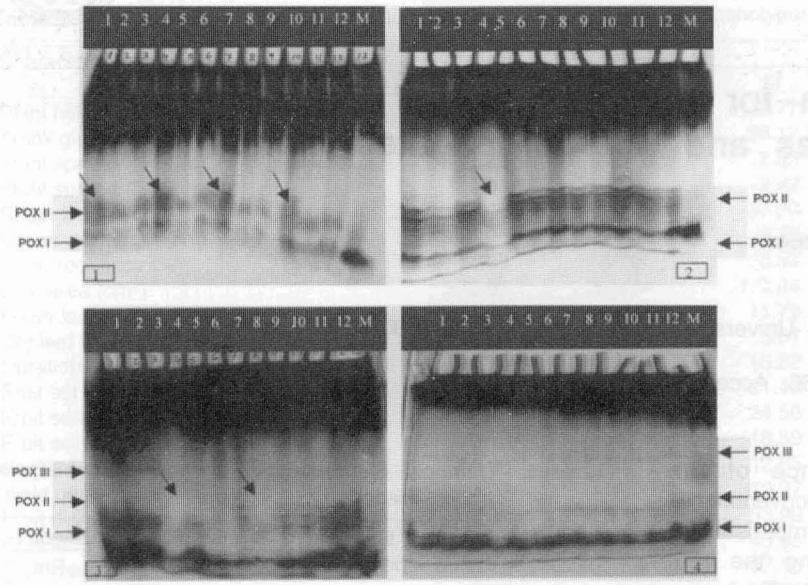

Fig. 1-4. Peroxidase zymogram in conventional and micropropagated clones of black pepper: (1) Panniyur 1, variant TC P_1-1 (well 1, 4, 7, 10); CC-well 3, 6, 9, 12; TCC-2, 5, 8, 11; M-marker; (2) Panniyur 2, variant TC P_2-7 (well 4); CC-well 11, 12; TCC-well 1 to 10; M-marker; (3) Panniyur 4, variant TC P_4-4 (well 4) and TC P_4-8 (well 8); CC-well 11, 12; TCC-well 1 to 10; M-marker; (4) TCC of Subhakara: All monomorphic; M-marker

bands in all the conventional and TC clones except for the two variants, TC P_4-4 and TC P_4-8 where there were only five bands. In Subhakara, the TC sample showed more number of fast moving as well as slow moving bands compared to clonal samples. There were four bands in the faster zone and seven bands in the slower zones in TC sample. On the other hand, there were only a total of six isozymes in conventional clones. Within TC clones all vines exhibited uniform pattern.

The inter-varietal polymorphism with respect to peroxidase showed two major zones of activity in the green leaf sheathed varieties *viz.*, P_1 and P_2 and three zones in the red leaf sheathed varieties *viz.*, P_4 and Subhakara. Among the 16 isozymes, PRX-I (1), PRX-I (5) and PRX-II (5) were monomorphic in all the varieties. PRX-I (1), the fastest among the 16 was comparatively with lesser intensity but was clear and well resolved in all the gels. PRX-I (5), the band corresponding to the marker band of *P. colubrinum* was the most prominent and intensely stained band in all varieties except in P_1. In the zone PRX-I, out of seven bands five showed varietal polymorphism. Among theses PRX-I (2) and PRX-I (3) were characteristic of P_2 whereas PRX-I (7) was characteristic of P_4 and TC Subhakara (Table 2). In the zone PRX-II there were eight isozymes of which, seven exhibited vareital polymorphism. But P_1 and P_2 shared five bands whereas P_4 and Subhakara shared two. PRX-II (4) was characteristic for Subhakara. The additional slow moving zone PRX-III with a single isozyme was specific for P_4 and TC Subhakara.

Among the four varieties P_2 had the largest number of isozymes, 13 out of the total 16 isoperoxidases were present in this variety. P_4 and TC plants of Subhakara showed more similar zymograms as they differed only with respect to PRX-II zone. So in general, it appeared that to a certain extent, P_1 and P_2 resembled each other whereas P_4 and Subhakara resembled each other with respect to isoperoxidase banding pattern. Based on the 10 isozymes with highest polymorphism genetic diversity analysis was carried out which showed that P_1 and P_2 were genetically more similar (GD = 0.23) whereas P_4 and Subhakara were closer (GD = 0.33). The extent of similarity was more in the former group. Comparison between the groups indicated that, from Subhakara, both P_1 and P_2 had equal distance (GD = 0.5) whereas from P_4, P_2 was more distant (GD = 0.53) than P_1 (GD = 0.42).

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References

1. **Hemaprabha G., Natarajan U. S. and Balasundaram N.** 2004. Relationship between isozyme diversity of parental clones and progeny performance in sugarcane (*Saccharam* spp.). Indian J. Genet., **64**: 9-12.

2. **Zaman A., Islam R. and Joarder O. I.** 1997. Field performance and biochemical evaluation of micropropagated mulberry plants. Pl. Cell Tissue Org. Cult., **51**: 61-64.

3. **Shaw C. R. and Koen P. L.** 1968. Starch gel zone electrophoresis of enzymes. *In*: Chromatogaphic and Electrophoretic Techniques Vol. 2 (ed. I. Smith), John Wiley, New York, USA: 325-359.

4. **Reyes L. M. C., Martinez O. W. and Beltran M.** 1998. Chemical variability in the genus *Musa*: Genetic characterisation using nine enzyme systems, Infomusa, **7**: 6-8.

5. **Alarmelu S., Balasubramaniam T. and Padmanabhan C.** 1999. Isozyme changes during morphogenesis in the *in vitro* cultures of *Panicum maximum*. Indian J. Genet., **59**: 47-52.

6. **Denieke T., Lynch D. R., Kawchuk L. M., Kozub G. C. and Armstrong J. D.** 1996. Genetic diversity of potato determined by random amplified polymorphic DNA analysis. Pl Cell Rep., **15**: 662-667.