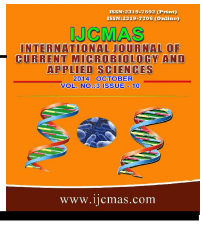
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 10 (2014) pp. 345-348**
http://www.ijcmas.com



Original Research Article

Variability in isoenzyme esterases and acid phosphatases pattern in different population of both the *Rheum* species from Garhwal Himalaya, India

U.C.Maithani[1*], M.Belwal[1] and M.C.Nautiyal[2]

[1]Department of Botany, Govt. P.G College Gopeshwar, Chamoli, India
[2]High Altitude Plant Physiology Research Centre, Srinagar (Garhwal), India
**Corresponding author*

A B S T R A C T

Keywords

Isoenzyme, genetic diversity, esterases, phosphatases,

The present investigation reveals that Acid phosphatase activity was found very low in *R. emodi* especially in KN population, whereas in *R. moorcroftianum* acid phosphatase had high activity in all the three populations *viz.*, TN, VF and HK. SDS- PAGE patterns of seed polypeptides were also observed as reproducible and indicating qualitative as well as quantitative differences in all the studied populations.

Introduction

Rheum (viz *R.emodi* and *R. moorcroftianum* of Polygonaceae), a perennial stout herbaceous genus commonly known as Rhubarb is well represented by about 10 species in the temperate and alpine region of Himalaya. Out of which only two species, namely *R. emodi* and *R. moorcroftianum* have been reported from Garhwal Himalaya (Anonymous, 1972).

Markert and Moller (1959) proposed the word enzyme for multiple molecular forms, sharing a catalytic activity derived from a tissue of a particular organism. Protein (enzyme) can be separated in to different molecular forms or isoenzymes by many biochemical methods (Shannon, 1968).

The gel electrophoresis is perhaps found to be the most versatile and easily applied to solve the critical problems in plant sciences. To know the genetic diversity of endangered species and their conservation have become in priority in recent years. Global genetic variability is also considered to be the basis for potential evolutionary changes in taxon and is believed to be insolence the overall physiological performance of these species.

Materials and Methods

Seeds of *R. emodi* and *R. moorcroftianum* collected from different regions of Garhwal Himalaya were used for isoenzyme analysis and SDS-PAGE protein profile, seeds were

soaked in distilled water for 24 hours. The seed coat of water soaked seeds was removed and 1 gm naked embryos, parts of the seeds or without seed coat were homogenized in 0.1 M trisHcl buffer containing 0.5% mercaptoethanol and 1% polyvinylpyrrolidone.

The samples were centrifuged at 10,000 rpm for 30 min and resulting supernatants were used for protein estimation. Protein content of each sample was determined according to Bradford method (Bradford, 1976).

Isoenzyme viz., esterases and acid phosphatases were visualized on 7.5% polyacrylamide slabgels using a discontinuous gel electrophoretic system. Equal quantity of protein was loaded on to the 7.5% polyacrylamide gel. The electrophoretic procedure was performed according to the method modified by Bhadula and Sawhney (1987).

Results and Discussion

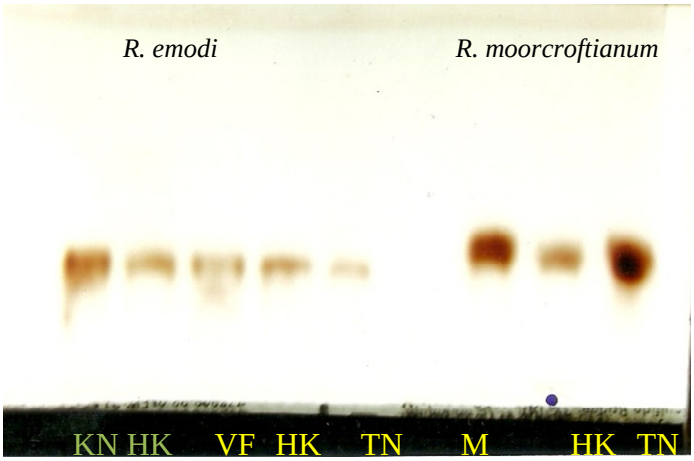
In the seeds of *R. moorcroftianum* acid phosphatase band patterns appearance was found dark in Tungnath population, followed by MD and HK. Whereas, in case of *R. emodi* the band intensity was observed as dark in TN in comparison to other populations. Light bands were appeared in all other populations of *R. emodi* and *R. moorcroftianum*, and the placement of band was also found to be similar among the different populations (plate 1a).

In *R. moorcroftianum* appearance of esterase band pattern was dark in all the three populations, viz., TN, HK, and MD while all the five populations of *R.emodi* showed light band pattern. In most of the population studied the number of band appearance in *R. emodi* was more than *R. moocroftianum*. The SDS PAGE inter population variation in different population of both the *Rheum* species were observed. In *R. moorcroftianum* appearance of polypeptide patterns was observed as dark in comparison to *R. emodi*.

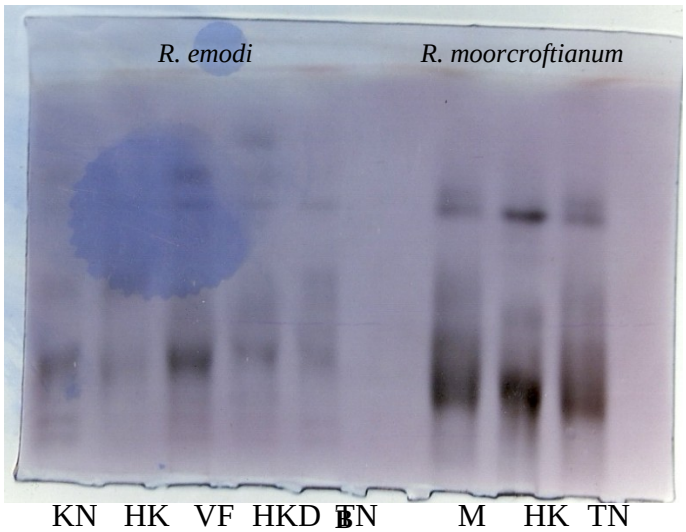
In case of *R. emodi* the maximum number of peaks (23) were recorded in TN populations followed by HKD and KN (22), and minimum were recorded in VF and HK (19). Similarly in *R. moorcroftianum* the maximum packs (18) were recorded in TN populations while minimum (17) were recorded in both HK and MD populations (Plate).

Polypeptide pattern and esterase isoenzymes showed considerable variability in different population of *R. emodi* and *R. moorcroftianum* although several common bands of polypeptides and esterases were present in *Rheum* seeds of all population, some of them to be specific and it showed the adaptation of these plants and different thermal regimes. Several isoenzymes including esterase have also been used in the analysis of genetic diversity of endangered species (Bousquenet et at; 1986; Godt and Hamrick 1995).

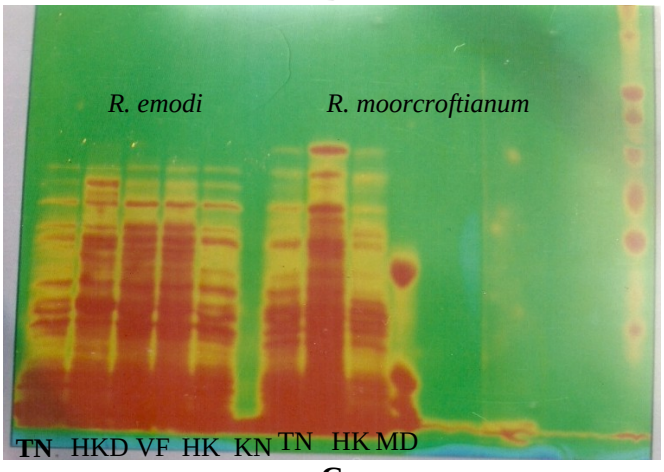
Acid phosphatase has been shown to increase during heat hardening and adaptive signifcance of this increased thermostability has also been reported (Feldman *et al.*, 1966, 1975).



A



B



C

Plate.1 Inter population variation in acid phosphatase (a) esterase (b) and polypeptide patterns of seeds of natural population of *R. emodi* and *R. moorcroftianum*

Acknowledgements

Authors are thankful to the Director, High Altitude Plant Physiology Research Center, Srinagar (Garhwal) Uttarakhand for extending facilities and chemical analysis.

References

Anonymous, (1972). The Wealth of India. Press and Information Directrorate CSIR, New Delhi Vol. ll, pp 3-6.

Bhadula, S.K., Sawhney, V.K. 1987. Esterase activity and isozymes during the ontogeny of stamens of male fertile Lycopersicon esculentum Mill., a male sterile stamenless –2mutant and low temperature – reverted mutant. *Plant Sci.,* 52: 187–194.

Bousquenet, J., Cheliak, W.M., Lalonde, M. 1986. Allozyme variability in natural populations of green alder (Alnus crispa) in Quebec. Genome, 29: 345–352.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Ann. Biochem.,* 72: 248–254.

Feldman, N.L., Kamentsera, I.E., Yurasheres Kaya, K.N. 1966. Acid phosphatase, thermostat bility m the extract of cucumber and wheat seedlings of ter heat hardening. In: Russ Tsitologiya, 8: 755–759.

Feldmans, N.L., Lutova, M.I., Scherbakava, A.M. 1975. Resistance of some proteins of *Pisum sativum* L. leaves after heat hardening to elevated temperature proteolysis and shifts. *pH. J. Therm Biot.,* 1: 47–51.

Godt, M.J.W., Hamrick, J.L. 1995. Allozyme variation in two great smoky mountain endemics: *Glyceria nubegena* and *Rugelia nudicalis*. *J. Hered.,* 86: 194–198.

Markert, C.L., Moller, F. 1959. Multiple forms of enzymes tissue ontogenetic and species specific patterns. *Proc. Nat. Acad. Sci.,* 45: 753–763.

Shannon, L.M. 1968. Plant isoenzyme. *Ann. Rev. Plant Physiol.,* 19: 187–210.