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Assessment of Biochemical and Molecular Diversity of Five Elite *Gladiolus* Varieties

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ABSTRACT

Five selected – based on market demand in the floricultural belt of West Bengal, India – *Gladiolus* (*Gladiolus hybridus* L.; family: Iridaceae) cultivars ('White Friendship' and 'Red Ginger' – hilly region cultivars and 'Green Bay', 'Intrepid' and 'Sabnam' – plateau cultivars) were characterized using biochemical (leaf phenolic profile by TLC and assessment of isozymes – esterase and acid phosphatase) and molecular (RAPD profile) markers. A dendrogram constructed following UPGMA revealed interrelationships among and/or between cultivars that could be explored for efficient breeding and improvement.

Keywords: genetic diversity, isozymes, RAPD profile, thin layer chromatography
Abbreviations: CTAB, cetyl trimethyl ammonium bromide; **dATP**, deoxy adenosine triphosphate; **dCTP**, deoxy cytosine triphosphate; **dGTP**, deoxy guanosine triphosphate; **dTTP**, deoxy thiamine triphosphate; **PAGE**, polyacrylamide gel electrophoresis; **PCR**, polemarase chain reaction; **RAPD**, random amplified polymorphic DNA; R_f, retention factor; **SOD**, superoxide dismutase; **Sol**, solvent; **TLC**, thin layer chromatography; **UPGMA**, unweighed pair group method with arithmetic mean

INTRODUCTION

Gladiolus (*Gladiolus hybridus* L.; family: Iridaceae) is an important commercial cut flower in floriculture (after rose) possessing a pivotal place both in domestic and international markets (Parthasarathy and Nagarajn 1999; Singh 2006; Singh *et al*. 2010). The modern cultivars of *Gladiolus hybridus* are believed to have originated from interspecific crosses among a number of wild species namely, *G. cruentus*, *G. natalensis*, *G. oppositiflorus*, *G. papilio* and *G. saundersii* (Barnard 1972; Imanishi 1989) thereby creating wide genetic variation in growth habit, size, shape, length of spikes and the colour of florets. An estimate of genetic diversity is useful to guide collection missions, monitoring genetic erosion, establishing core subsets and developing on-farm conservation strategies.

Biodiversity International is currently involved in conservation of *Gladiolus* cultivars (Ranjan *et al*. 2010), and such endeavors will be helpful in ascertaining unambiguous discrimination between accessions, identification of plant resources, detection of redundancies and also monitoring genetic changes appearing during maintenance. The present communication describes genetic interrelationship among and/or between some selected (based on market survey; Saha 2011) cultivars ('White Friendship' and 'Red Ginger' – hilly and red alluvial zone of West Bengal, temperature: 7°C min, 25°C max; 'Green Bay', 'Intrepid' and 'Sabnam' – Southern part of Bengal, deltic alluvial soil, temperature: 11.2-15.1°C min, 32-36°C max) of *Gladiolus* following the use of biochemical (thin layer chromatography (TLC) of phenols in leaves and isozyme analysis) and molecular (detection of polymorphism using RAPD primers) markers with the objective of characterizing these cultivars and generating information which may be crucial in guiding an

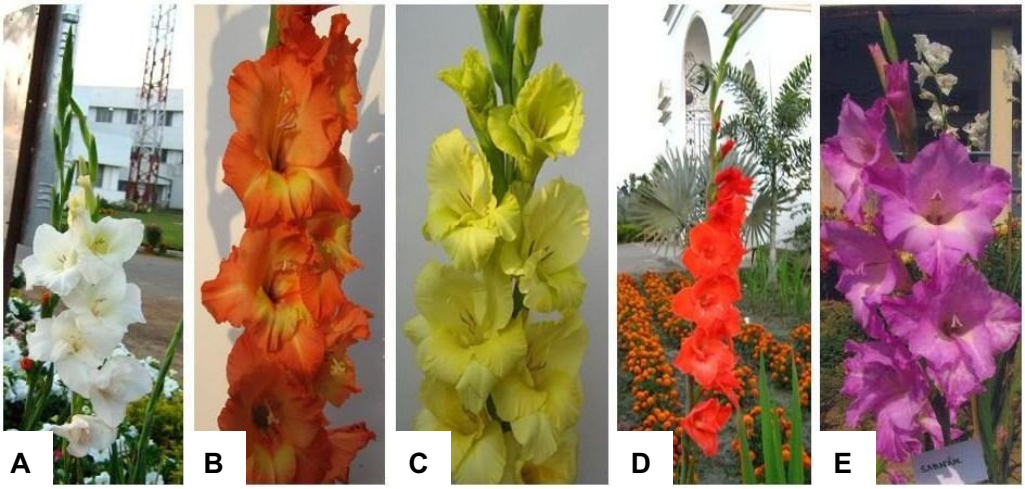

Fig. 1 *Gladiolus* cultivars used in this study. (**A**) 'White Friendship'; (**B**) 'Red Ginger'; (**C**) 'Green Bay'; (**D**) 'Intrepid'; (**E**) 'Sabnam'.

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improvement (efficient breeding) and conservation programme.

MATERIALS AND METHODS

Plant material

The corms of five *Gladiolus* cultivars ('White Friendship' – traffic white, 9016; 'Red Ginger' – red orange, 2001; 'Green Bay' – yellow, 1016; 'Intrepid' – luminary red, 3024; 'Sabnam' – heather violet, 4006; RAL classic colour chart, UK, **Fig. 1A-E**) were collected from the different cultivation zones of West Bengal. The cultivars could be easily identified from the colour of their florets. The corms were cultivated and maintained in the experimental garden of Haldia Institute of Technology (22° 03′ N: latitude; 88° 04′ E: longitude; 6 m: altitude) at Haldia, Purba Medinipur, West Bengal, India.

Thin layer chromatography

For the TLC study of phenolic compounds in leaves, five plants of each cultivar were selected and 2 g of young leaves were extracted separately with 2 ml of methanol containing 1% hydrochloric acid. The chromatograms were developed on glass plates (14 × 14 cm) coated with cellulose powder (Sigma Cell Type-20). The plates were then run in one direction (10 cm) in 1% formic acid (solvent I) and, after drying in air, they were run in the second direction (12 cm) in a mixture of benzene: propionic acid: water (20: 42: 10, v/v; solvent II). The plates were dried and sprayed with 1% flavones reagent (diphenyl boric acid ethanol-amine complex, Sigma) in methanol. The spots that were observed were marked under long wave UV light (360 nm). The mean retention factor (R_f) values in both directions for each spot were calculated from five good plates for each sample. R_f values were transformed to hR_f ($R_f \times 100$) values.

Isoenzyme analysis

Enzymes (proteins) were extracted according to Wetter and Dyck (1983). Leaf tissues from sprouted corms (2 g) of each cultivar were extracted, homogenized with 5 ml 0.2 M Tris-HCl buffer (pH 8.5) containing 0.056 M 2-mercaptoethanol and 1 M sucrose, and centrifuged at 15,000 ×*g* for 20 min at 4°C. Polyacrylamide gel electrophoresis (PAGE) of the supernatant was carried out in slab gels with a separating gel of 10% acrylamide (pH 8.8), an upper stacking gel of 3% acrylamide (pH 6.9) and Tris-Glycine (pH 8.3) running buffer. The two enzymes whose isozyme polymorphism was examined by PAGE were esterase (EST; E.C. 3.1.1.1) and acid phosphatase (ACP; E.C. 3.1.3.2). Gels were stained following the methods of Das and Mukherjee (1997). Differential distribution of isozyme bands in the five *Gladiolus* cultivars was visually assessed.

Detection of polymorphism using RAPD primers

DNA was extracted – for all 5 cultivars – from young leaves of plants derived from *in vitro* corms by using a modified CTAB method (Rogers and Bendich 1988). The RAPD reaction for each sample was performed following Williams *et al*. (1990). Ten olegonucleotide primers (Operon Technologies, Alameda, USA; OPB-01-OPB-10) were used as these were previously shown to be successful in detecting polymorphism in *Gladiolus* (Roy *et al*. 2006). Amplifications were carried out in a thermal cycler (Perkin Elmer System-2400, Norwalk, CT, USA) with an initial denaturation of 120 sec at 94°C. The temperature profile of each cycle was 60 sec denaturation at 94°C, 60 sec annealing at 35°C and 120 sec extension at 72°C. The reaction was performed for 45 cycles followed by 300 sec hold at 72°C to ensure completion of primer extension. The PCR reaction mixture (25 µl) consisted of 1X buffer, 0.2 mM dATP, dCTP, dGTP, dTTP, 2 mM $MgCl_2$, 0.2 µM of primer, 100 ng of template DNA and 1U of *Taq* DNA polymerase (Roche, Indianapolis, USA). Amplified products were electrophoresed on a 1.8% agarose gel with DNA molecular weight marker ØX174 *Hae*II digest (GENE India Pvt. Ltd., Bangalore) as the molecular size marker.

Table 1 Colour and hR_f of spots present in phenolic extraction (from leaf) of five *Gladiolus* cultivars.

Spot No.	Colour	hR_f Sol. I	hR_f Sol. II	A	B	C	D	E
1	O	36	25	+	+	+	+	+
2	YG	36	42	+	+	+	+	+
3	YG	53	51	+	+	+	+	+
4	BW	52	73	+	+	+	+	+
5	B	62	75	+	+	+	+	+
6	BW	14	47	+	-	+	+	-
7	BW	14	78	+	+	+	+	+
8	BW	62	0	-	-	+	-	-
9	BW	12	18	+	+	-	+	+
10	R	6	31	-	-	+	-	-
11	BW	10	41	-	+	-	-	-
12	YG	67	51	+	+	-	+	+
13	B	83	78	-	+	-	-	-
14	YG	90	12	-	+	-	-	-
15	R(Vis)	82	16	-	+	-	-	-
16	YG	86	52	-	-	+	-	-
17	YG	59	60	+	+	-	+	+
18	BW	30	71	-	-	-	+	-
19	BW	19	76	-	-	-	+	-
20	YG	58	88	-	-	-	+	-

A = 'Green Bay'; B = 'Intrepid'; C = 'Sabnam'; D = 'White Friendship'; E = 'Red Ginger'; O = orange; YG = yellow green; BW = blue white; B = blue, R = red, R(Vis) = Red visible without spray; '+' = presence of spot; '-' = absence of spot; hR_f = R_f 100

Statistical analysis

Cluster analysis (using software STASTICA 4.5) based on a proximity matrix (using variables obtained from the leaf phenolic profile, isozyme and RAPD analysis) was made by the unweighed pair group method for arithmetic average (UPGMA) and a corresponding dendrogram was constructed using IBM SPSS statistics (V. 19, 2010) software.

RESULTS AND DISCUSSION

Phenolic profile analysis of the selected cultivars

TLC data (**Table 1**) revealed 10 spots in 'White Friendship' (nos. 1 to 7, 12 and 17) 12 (nos. 1-5, 7, 10, 12-15, 17) in 'Red Ginger', 9 (nos.1-9) in 'Green Bay', 13 (nos. 1-7, 10, 12, 17, 19, 20, 21) in 'Intrepid' and 11 (nos. 1-5, 7, 10, 12, 15, 17, 21) in 'Sabnam'. 'Intrepid' showed 3 new spots. The hR_f of the spots and their differential colorations were noted.

Isoenzyme analysis

Banding profiles of EST (common bands: R_f 0.13, 0.41, 0.47; **Fig. 2A, 2B**) and ACP (common bands: R_f being 0.08, 0.11, 0.12, 0.35, 0.38 **Fig. 2C, 2D**) showed few common bands among the cultivars; however, polymorphism was also noted. Lou *et al*. (2004) noted polymorphism with SOD (Superoxide dismutase) in different cultivars of *G. hybridus*. Heun *et al*. (1994) suggested isozyme markers to be efficient for determining genetic relationships among the breeding lines, varieties and different populations of *Avena sterilis*. Oleo *et al*. (1986) analyzed interspecific hybrids of *Beta* and revealed that due to the codominant expression of isozymes the effect of the combination of various sources of genetic information in an organism could be assessed.

RAPD profile

Out of the 10 oligonucleotide primers used, no detectable amplification was obtained for two primers (OPB-06 and OPB-10). From the remaining eight decamer primers, a total of 91 fragments were amplified, inclusive of monomorphic bands in all the samples (**Fig. 3A-3F**). Most of the bands were < 1500 bp, but > 200 bp. The number of bands generated by each primer ranged from 7 (OPB-7) to 18 (OPB-9) with a mean of 11 bands/primer. Out of 91 bands,

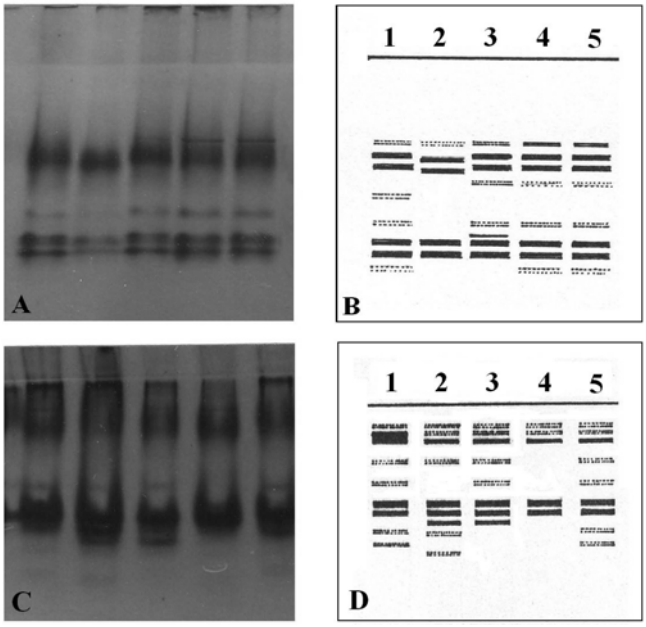

Fig. 2 Isozymic profiles and zymograms. (**A**) Esterase (EST; E.C. 3.1.1.1) and (**C**) Acid phosphatase (ACP; E.C. 3.1.3.2) profiles of the five *Gladiolus* cultivars (1: 'White Friendship'; 2: 'Red Ginger'; 3: 'Green Bay'; 4: 'Intrepid'; 5: 'Sabnam'). Zymograms are a schematic representation of the band profile of isoenzymes; (**B**) represents EST and (**D**) represents ACP.

54 (59%) were polymorphic. The primer showing the maximum number of polymorphic bands was OPB-9 (14 bands) followed by OPB-4 (10 bands). Primers OPB-9 and OPB-4 most efficiently detected genetic diversity in *Gladiolus*. Sarkar *et al*. (2010) reported that RAPD primers with maximum polymorphic loci possess a higher marker index with high resolving power. Takatsu *et al*. (2001) reported the effective use of RAPD markers in wild *Gladiolus* germplasm for detecting genetic relationships.

Phylogenetic relationships of the selected cultivars based on allelic polymorphisms

On the basis of TCL, isozyme and RAPD analyses among the five *Gladiolus* genotypes, UPGMA analysis conducted on proximity matrix (polymorphism ranged from 0.450 to 5.916) revealed two major clusters or groups (**Fig. 4**). The first cluster consisted of 'Red Ginger' while the other incorporated the remaining four cultivars. 'White Friendship' and 'Sabnam' are distinctly related between themselves. Relative closeness among cultivars can be explored for breeding improvement programme.

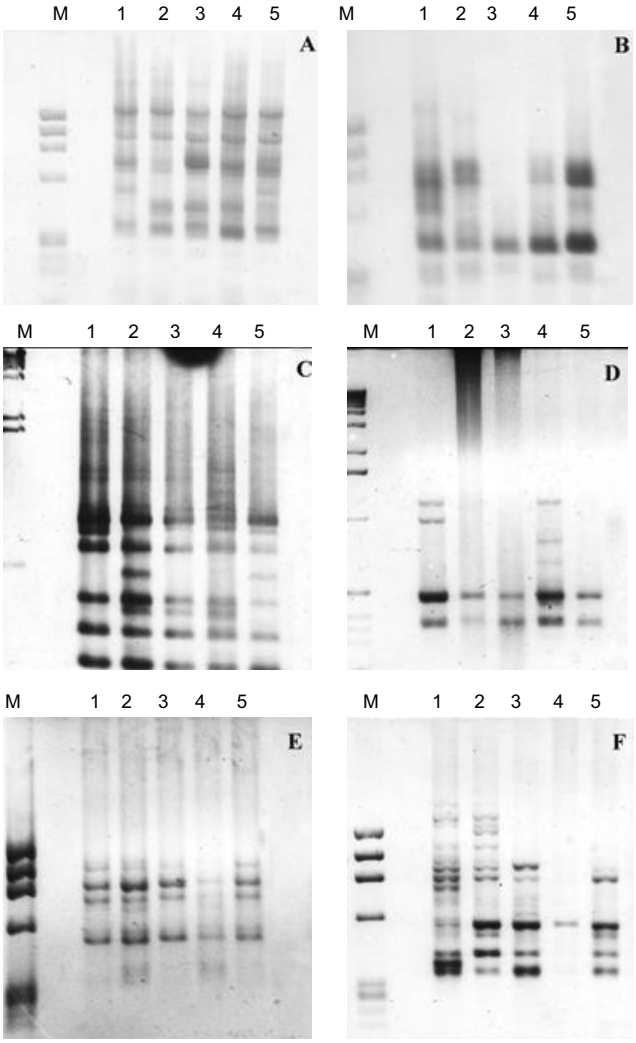

Fig. 3 RAPD profiles of selected *Gladiolus* cultivars. 1: 'White Friendship'; 2: 'Red Ginger'; 3: 'Green Bay'; 4: 'Intrepid'; 5: 'Sabnam'. Six RAPD primers are shown: (**A**) OPB-2; (**B**) OPB-3; (**C**) OPB-4; (**D**) OPB-7; (**E**) OPB-8; (**F**) OPB-9. M: DNA molecular weight marker (ØX174 *Hae*II digest).

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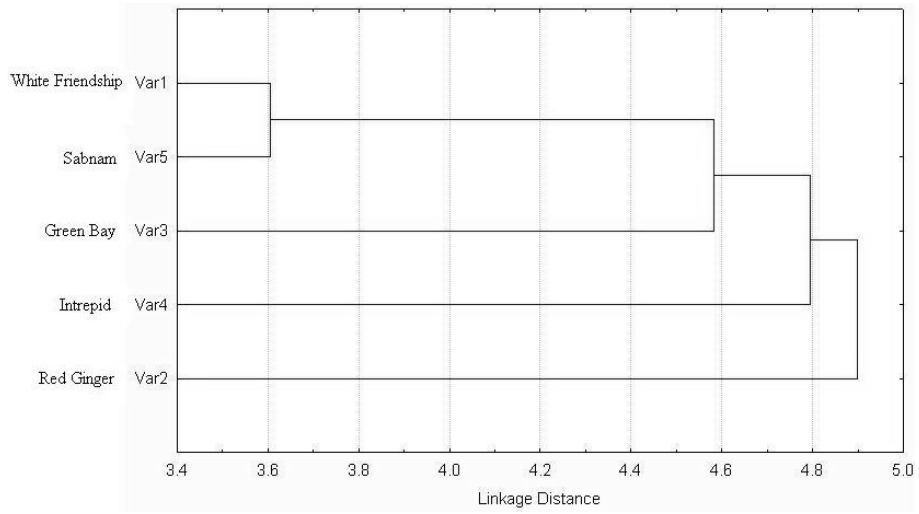

Fig. 4 Dendogram showing clustering of five *Gladiolus* cultivars following UPGMA. 1: 'White Friend-ship'; 2: 'Red Ginger'; 3: 'Green Bay'; 4: 'Intrepid'; 5: 'Sabnam'.