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RESEARCH ARTICLE

Identification of Physiological and Biochemical Markers for Rapid Testing of Hybrid Purity in Maize (*Zea mays*) Hybrids during Seedling Stage

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Abstract

The present study aimed to identify physiological and biochemical markers to assess the hybrid purity of two maize (*Zea mays* L.) hybrids, PMH 13 and PMH 14, released by Punjab Agricultural University in 2021 and 2023, respectively. Seeds of the maize hybrid PMH 13, along with its female parent (LM 27) and male parent (LM 17), as well as seeds of hybrid PMH 14 and its female parent (LM 28) and male parent (LM 29), were obtained from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Both hybrids, PMH 13 and PMH 14, exhibited significantly higher germination rates, superior seedling length, increased dry weight and elevated SVI I and II values, accompanied by a reduced mean germination time compared to their respective parental lines. Zymograms of superoxide dismutase and esterase revealed polymorphism and thus can be employed to test PMH 13 and PMH 14 hybrid purity. Zymography of peroxidase can be utilized to detect selfed female seed (off types) in PMH 14. Protein profiling of total soluble proteins revealed one polymorphic polypeptide band in the hybrid corresponding to a polypeptide of 29 kDa in female and 30 kDa in male, which can be used for confirming the hybrid purity of maize hybrid PMH 13. No polymorphic polypeptide band was observed in hybrid PMH 14. Thus, SDS-PAGE analysis of total soluble proteins cannot be used for testing the hybrid purity of PMH 14 from its off types.

Keywords: Esterase, Hybrid, Isozyme, Maize, Polymorphism

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Introduction

Maize (*Zea mays* L.), belonging to the Poaceae family, often known as "Miracle Crop" and the "Queen of Cereals", is the third most significant cereal crop in the world, following rice and wheat. Major maize-growing countries are Brazil, China, India, Indonesia, Mexico, the Philippines, South Africa and the USA. Globally, the crop occupies approximately 205 million hectares, with production reaching nearly 1.21 billion metric tonnes during 2023-24 (FAOSTAT 2025).

The introduction of new high-yielding hybrids at the commercial level has contributed significantly to the rise in maize productivity. However, maintaining genetic purity during multiplication stages is challenging and susceptible to contamination from factors such as the presence of pollen shedders, cross-pollination with foreign pollen, and physical admixtures. Using seeds of low genetic purity can lead to genetic deterioration of hybrids, segregation of traits and lower yields. Therefore, genetic purity tests are essential to verify any deviations from the original hybrid during multiplication (Sherakhane *et al*., 2025).

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Two kinds of genetic markers are being used to determine hybrid purity: classical markers and molecular markers (Elci and Hancer, 2014). There are three types of classical markers: morphological, cytological, and biochemical markers. Traditionally, the purity of hybrids is assessed using morphological markers through a grow-out test (GOT) in the field. However, in GOT, plants are grown up to the maturity stage for identification of off-types through assessing various morphological as well as floral characteristics. But it is time-consuming (needs one full season for completion), resource-intensive, space-demanding, and influenced by environmental factors (Garg *et al*., 2026).

Cytological markers involve studying chromosome number and structure through microscopic examination, require specialized equipment and are less practical for large-scale testing. To overcome these limitations, biochemical markers are employed for hybrid purity testing, offering the advantage of evaluating multiple loci simultaneously on a single gel. Additionally, the analysis process requires only a small amount of plant tissue and sample extract (Rakshit *et al.,* 2011). On an electrophoretic gel, isozymes are visible when they react with particular activity stains, allowing for the detection of specific enzymes by providing appropriate substrates and cofactors required for activity in the staining solution (Rivoal *et al.,* 2002).

Isozymes, which are direct products of gene expression, display variations in protein-coding genes and arise from multiple gene loci that code for structurally distinct polypeptide chains (Huqail and Abdelhaleim, 2015). To determine the genetic purity of maize cultivars, a high-resolution polyacrylamide gel electrophoresis (PAGE) technique that examines polymorphism in salt-soluble proteins has been utilized (Wang *et al.,* 1994). Rakshit *et al.,* (2011) utilized four isozyme markers-esterase, acid phosphatase, alcohol dehydrogenase, and malic dehydrogenase to differentiate between six maize hybrids. Sanghamitra *et al*., (2017) used four isozymes *viz*., alcohol dehydrogenase, esterase, acid phosphatase and malate dehydrogenase, to evaluate the genetic purity of two maize hybrids.

Physiological studies conducted on seed vigour in two maize hybrids (SHM-1 and DHM-117) and their parental lines revealed that germination rate and seedling length were much higher in hybrids compared to the parental lines (Kumar *et al.,* 2021). Mula *et al.,* (2015) reported that the pigeonpea hybrids exhibited significantly higher germination rates, longer radicle lengths, higher seedling dry weights and greater seedling vigour index II compared to the varieties of pigeon pea.

This study was conducted to identify physiological and biochemical markers at the seedling stage for the rapid assessment of hybrid purity in two maize hybrids, PMH 13 and PMH 14, as an alternative to the conventional method of testing hybrid purity utilizing DUS characterization that lacks the resolving power to identify hybrid purity at seedling or juvenile stages but requires the crop to enter into the reproductive stage.

Materials and Methods

The present study was carried out in the Seed Physiology Laboratory, Office of the Director (Seeds), Punjab Agricultural University, Ludhiana, during 2022-2024. Seeds of the maize hybrids PMH 13 and PMH 14, along with their respective parents, female parent LM 27 and male parent LM 17 of PMH 13, and female parent LM 28 and male parent LM 29 of PMH 14, were sourced from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana.

Physiological markers

In accordance with the International Seed Testing Association (2021), the germination studies were carried out in the laboratory by the "between paper" method at 25±2°C with 100 seeds uniformly arranged on a moistened germination sheet covered by another germination sheet and wrapped in wax paper for moisture retention and placed upright in an incubator for seven days. On the 7th day, the final data on germination were recorded, and germination percentage was calculated as:

$$\text{Germination (\%)} = [\text{No. of seeds germinated/total seed number}] \times 100$$

Ten representatives of seven-day-old seedlings were evaluated for the vigour index by recording root and shoot length and dry weight of seedlings. The vigour indices were estimated in accordance with Abdul-Baki and Anderson (1973) as:

SVI I = Germination (%) × average seedling length (cm)

SVI II = Germination (%) × dry weight of ten seedlings (g)

To calculate germination speed, seeds were placed on moist double blotting paper in Petri dishes and incubated in a BOD incubator at 25±2°C for 7 days. The number of seeds germinated each day was recorded, and the speed of germination was calculated according to the method described by AOSA (Association of Official Seed Analysts, 1983) as:

$$\text{Speed of germination} = \{ \frac{n1}{d1} + \frac{n2}{d2} + \frac{n3}{d3} + \text{————} \}$$

where, n = number of seeds germinated

d= number of days

Mean germination time was calculated as described by Ellis and Roberts (1981):

$$MGT = \{ \frac{n1 \times d1 + n2 \times d2 + n3 \times d3 + \text{--------}}{\text{Total no. of seeds germinated}} \}$$

where n, number of seeds germinated; d, number of days.

Statistical analysis: Data related to germination percentage, speed of germination, mean germination time, average seedling length, dry weight of ten seedlings, and seedling vigour index I and II were analyzed using analysis of variance (ANOVA) by Tukey's test.

Biochemical markers

Native-Polyacrylamide Gel Electrophoresis for iso-enzymes: The isoenzymes were isolated from the shoots of seven-day-old seedlings. Homogenization of seedlings was carried out in 2 ml of potassium phosphate buffer with pH 7.5, and centrifugation of the resulting homogenate was carried out under low-temperature conditions (4°C) at 10,000 rpm for 25 minutes. The enzyme supernatant was collected, and its protein concentration was estimated by the protocol given by Lowry *et al.,* (1951). Then, the enzyme supernatant was mixed with the sample buffer and loaded onto the gel. A gel consisting of two components-stacking gel (4%, pH 6.8) and resolving gel (12%, pH 8.8) was prepared as described by Laemmli (1970) in a large-format vertical gel electrophoresis system (Model TV400Y, SCIE-PLAS Ltd.). Electrophoresis was initially run at 90V for 15 minutes, followed by 180V until the tracking dye reached the bottom of the gel. After the electrophoresis run, the gel was stained using various staining procedures. The staining procedure given by Wilkesman *et al.,* (2014) was followed for peroxidase (POD), Spitz and Oberley (2001) for superoxide dismutase (SOD) and Brewbaker *et al.,* (1968) for esterase (EST). For every band, relative mobility values were calculated. A gel photograph was captured, and zymograms were drawn to scale.

Staining procedure for POD: The gel was rinsed with distilled water and then incubated in a 1% Triton solution for 30 minutes. The solution was prepared by combining 1.5 ml of guaiacol with 15 ml of ethanol. This solution was then mixed with 200 ml of 50 mM phosphate buffer (pH 7) containing 2.5 ml of 0.3% hydrogen peroxide. Subsequently, the gel was immersed in this solution and incubated in the dark for approximately 20 minutes until reddish-brown bands started to develop. Once the bands were sufficiently stained, the gel was immersed in 7% acetic acid for 10 minutes.

Staining procedure for SOD: The gel was soaked for 20 minutes in 200 ml chilled distilled water containing 0.28 mM nitrobluetetrazolium (NBT). 200 ml of 36 mM potassium phosphate buffer (pH 7.8) containing 850 µl TEMED and 0.0021 g riboflavin was prepared, and the gel was immersed in the solution and kept in the sunlight for about 10 minutes till the appearance of white-coloured bands against a brownish background.

Staining procedure for EST: A 1% naphthyl acetate solution was prepared by dissolving 0.1 g of naphthyl acetate in 10 ml of 70% ethanol. Separately, 0.5 g of fast blue RR salt was dissolved in 200 ml of phosphate buffer (pH 7). After thoroughly mixing the solutions, the gel was incubated in the staining solution at 37°C until brownish-black bands appeared.

Electrophoresis of total soluble proteins by SDS-PAGE: Total soluble proteins were extracted from 300 mg of ground seeds from the hybrids PMH 13 and PMH 14 and their parent lines using 2 ml of extraction buffer. The buffer consisted of 1% sodium dodecyl sulphate (SDS), 1% mercaptoethanol, 0.25 M sodium chloride and 0.05 M sodium phosphate (monobasic). The seed extract was centrifuged at 10,000 rpm for 20 minutes at 4°C and the supernatant was carefully collected, avoiding the top lipid layer. The supernatant was then centrifuged again at 10,000 rpm for 10 minutes at 4°C. The final supernatant was collected and stored at -20°C until further use. The method suggested by Lowry *et al.,* (1951) was used to estimate total soluble proteins. An aliquot of supernatant containing 90 µg of protein was loaded into each well in a large-format vertical gel electrophoresis system (Model TV400Y, SCIE-PLAS Ltd.). Protein samples were mixed with an equal volume of sample buffer and then incubated at 95°C for 3 minutes. A gel consisting of two components-stacking gel (4%, pH 6.8) and resolving gel (12%, pH 8.8) was prepared as described by Laemmli (1970). The samples, along with a known molecular weight marker ranging from 20 to 220 kDa, were loaded into wells. Electrophoresis was initially performed at 90V for 15 minutes, then the voltage was increased to 180V and continued until the dye reached the bottom of the gel. After electrophoresis, the gel was placed in 12.5% trichloroacetic acid solution for prefixing the resolved proteins. The gel was then immersed in a staining solution containing 0.5 g of Coomassie Brilliant Blue

and 5 ml of methanol in 500 ml of destaining solution for 2 hours. After staining, the gel was destained by immersing it in a solution of glacial acetic acid, methanol and distilled water in a ratio of 80:24:220 for approximately 3 hours. The gel was then photographed and analyzed using Gel Analyzer 23.1.

Results and Discussion

Physiological markers

The germination percentage, germination speed, average seedling length, seedling vigour index I (SVI I), dry weight of seedlings and seedling vigour index II (SVI II) were all significantly higher in the hybrid PMH 13 compared to both of its parent lines. Mean germination time was significantly lower in the hybrid compared to both parents; however, no significant difference was observed between the male and female parents. The female parent (LM 27) exhibited higher values of average seedling length, SVI I and SVI II as compared to the male parent (LM 17) (Table 1). Likewise, germination percentage of hybrid PMH 14 was significantly higher than both its parents. However, there was no significant difference in germination percentage between the female parent (LM 28) and the male parent (LM 29). The average seedling length, SVI I and SVI II were all significantly greater in the hybrid PMH 14 compared to both of its parent lines. Speed of germination exhibited the highest value in hybrid PMH 14, followed by the female parent (LM28) and male parent (LM 29), respectively. Conversely, hybrid PMH 14 exhibited the lowest value of mean germination time, followed by the female parent and male parent, respectively (Table 2). Mula *et al*., (2015) and Kumar *et al*., (2021) also recorded higher germination rate and seedling vigour in hybrids of pigeon pea and maize as compared to varieties and their parental lines, respectively.

Biochemical markers

Zymography of superoxide dismutase: Five isozyme bands of superoxide dismutase with Rm values ranging from 0.357 to 0.553 were observed in maize hybrid PMH 13 and its parents. Four bands with Rm values of 0.357, 0.446, 0.535 and 0.553 were commonly shared by hybrid PMH 13 and both its parents. The band with Rm value 0.401 was present in both parents but absent in the hybrid; thus, its absence can be used as a marker for confirming the hybrid purity of PMH 13 seed lots. In hybrid PMH 14 and its parents, LM 28 and LM 29, a total of five isozyme bands were observed. Three common bands corresponding to Rm values of 0.357, 0.535 and 0.553 were observed in hybrid PMH 14 and both its parents. Two bands having Rm values of 0.401 and 0.446 were found polymorphic in PMH 14. A band with Rm value of 0.401 was present in the male parent (LM 29) and the hybrid (PMH 14) but was absent in the female

Table 1: Seed quality parameters of hybrid PMH 13 and its parents

Parameter	PMH 13	LM 27 (Female parent)	LM 17 (Male parent)
Germination (%)	96.00^{a}	85.33^{b}	83.33^{b}
Speed of germination	3.13^{a}	2.69^{b}	2.62^{b}
Mean germination time (days)	5.36^{b}	6.50^{a}	6.22^{a}
Average seedling length (cm)	49.00^{a}	31.70^{b}	19.43^{c}
Seedling vigour index I	4703.60^{a}	2704.90^{b}	1619.40^{c}
Dry weight of ten seedlings (g)	0.93^{a}	0.47^{b}	0.35^{c}
Seedling vigour index II	89.66^{a}	41.40^{b}	29.92^{c}

Mean values followed by different superscripts were significantly different (P<0.05) using Tukey's test.

Table 2: Seed quality parameters of hybrid PMH 14 and its parents

Parameter	PMH 14	LM 28 (Female parent)	LM 29 (Male parent)
Germination (%)	98.33^{a}	83.33^{b}	88.33^{b}
Speed of germination	3.43^{a}	2.63^{b}	2.32^{c}
Mean germination time (days)	5.43^{c}	6.23^{b}	7.21^{a}
Average seedling length (cm)	43.76^{a}	27.36^{c}	38.10^{b}
Seedling vigour index I	4304.20^{a}	2280.30^{c}	3365.30^{b}
Dry weight of ten seedlings (g)	0.90^{a}	0.40^{c}	0.52^{b}
Seedling vigour index II	88.64^{a}	33.33^{c}	46.48^{b}

Mean values followed by different superscripts were significantly different (P<0.05) using Tukey's test.

Table 3: Characterization of superoxide dismutase (SOD) isozyme bands along with Rm value in maize hybrids and their parents

Isoenzyme band	Rm value	LM 27	LM 17	PMH 13	LM 28	LM 29	PMH 14
1	0.357	+	+	+	+	+	+
2	0.401	+	+	-	-	+	+
3	0.446	+	+	+	+	-	+
4	0.535	+	+	+	+	+	+
5	0.553	+	+	+	+	+	+

parent, whereas another band of Rm value of 0.446 was present in the female parent (LM 28) and the hybrid but absent in the male parent. So, the SOD zymogram can be used for testing the purity of maize hybrid PMH 14 seed lots (Table 3, Fig. 1A and B).

Zymography of peroxidase: Maize hybrids PMH 13 and PMH 14 revealed a total of three isozyme bands of peroxidase with Rm values ranging from 0.047 to 0.257. The bands with Rm values of 0.047 and 0.123 were commonly shared by both maize hybrids and their parents. One unique isozyme band corresponding to Rm value of 0.257 was observed only in the male parent (LM 17) but was absent in the female parent and hybrid PMH 13. Thus, this band can serve as a unique marker to identify male parent LM 17. Isoenzyme band corresponding to Rm value of 0.257 was present in both female parent (LM 28) and hybrid PMH 14 but was absent in male parent (LM 29). Thus, zymography of the POD isoenzyme can serve as a maternal marker to detect selfed female seed (off-types), if any, in PMH 14 seed lots (Table 4, Fig. 2A and B).

Zymography of esterase: A total of six isozyme bands of esterase with Rm values ranging from 0.113-0.521 were observed in maize hybrid PMH 13 and its parents. Three isozyme bands corresponding to Rm values 0.113, 0.434 and 0.521 were commonly shared by maize hybrid PMH 13 and both its parents. Three polymorphic bands were observed in hybrid PMH 13 corresponding to bands with Rm values of 0.321 and 0.400, shared with the female parent LM 27 and a band with Rm value of 0.373, shared with the male parent LM 17. Apparently, zymography of EST can be used to confirm hybrid purity of PMH 13 seed lots. PMH 14 and its parents exhibited a total of seven isozyme bands with Rm values ranging from 0.130 to 0.521. Three

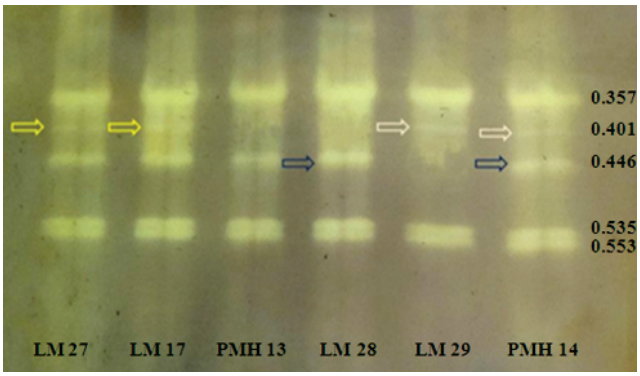


Fig. 1A: Zymography of superoxide dismutase (SOD) using nitroblue tetrazolium (NBT). Total distance travelled by the dye front is 11.2 cm.
LM 27, female parent of hybrid PMH 13; LM 17, male parent of hybrid PMH 13; PMH 13, maize hybrid; LM 28, female parent of hybrid PMH 14; LM 29, male parent of hybrid PMH 14; PMH 14, maize hybrid.

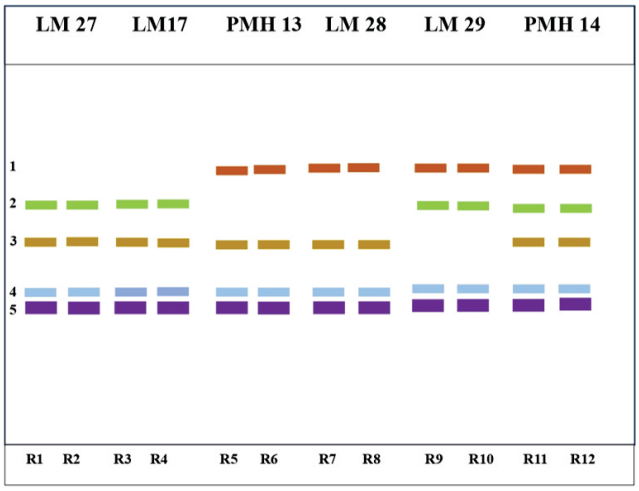


Fig. 1B: Diagrammatic representation of isozyme bands of SOD of two maize hybrids and their parents.
LM 27, female parent of hybrid PMH 13; LM 17, male parent of hybrid PMH 13; PMH 13, maize hybrid; LM 28, female parent of hybrid PMH 14; LM 29, male parent of hybrid PMH 14; PMH 14, maize hybrid.

Table 4: Characterization of peroxidase (POD) isozyme bands along with Rm value in maize hybrids and their parents

Isoenzyme band	Rm value	LM 27	LM 17	PMH 13	LM 28	LM 29	PMH 14
1	0.047	+	+	+	+	+	+
2	0.123	+	+	+	+	+	+
3	0.257	-	+	-	+	-	+

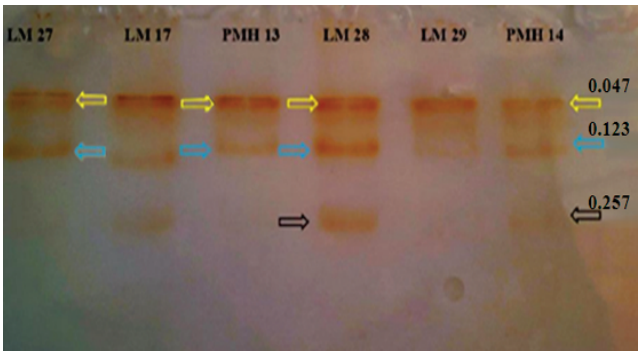


Fig. 2A: Zymography of peroxidase (POD) stained with guaiacol as the substrate.
The total distance travelled by the dye front is 10.5 cm.
LM 27, female parent of hybrid PMH 13; LM 17, male parent of hybrid PMH 13; PMH 13, maize hybrid; LM 28, female parent of hybrid PMH 14; LM 29, male parent of hybrid PMH 14; PMH 14, maize hybrid.

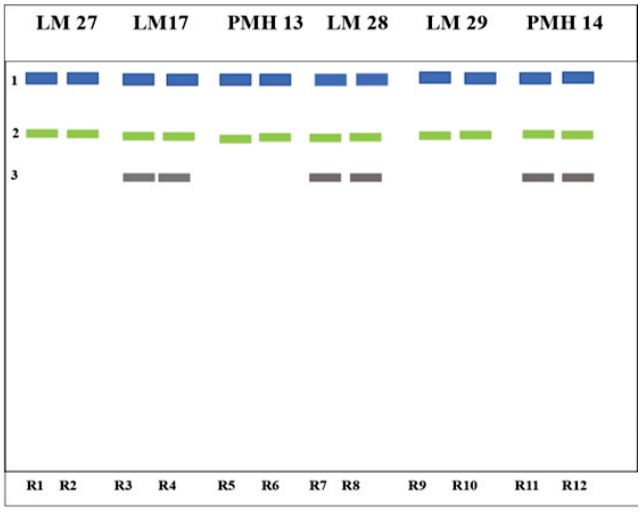


Fig. 2B: Diagrammatic representation of isozyme bands of POD in two maize hybrids and their parents.
LM 27, female parent of hybrid PMH 13; LM 17, male parent of hybrid PMH 13; PMH 13, maize hybrid; LM 28, female parent of hybrid PMH 14; LM 29, male parent of hybrid PMH 14; PMH 14, maize hybrid.

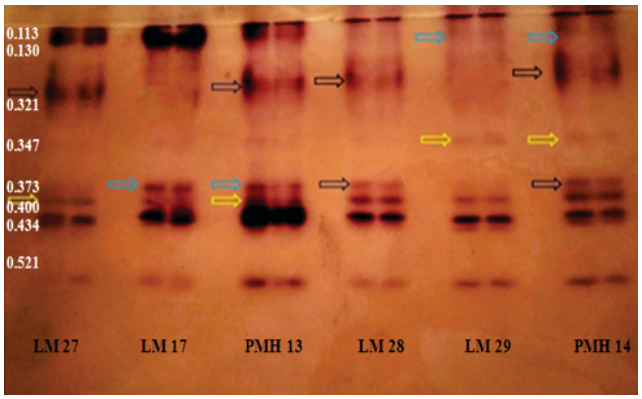


Fig. 3A: Zymography of esterase (EST) stained with naphthyl acetate and RR salt.
Total distance travelled by the dye front is 11.5 cm.
LM 27, female parent of hybrid PMH 13; LM 17, male parent of hybrid PMH 13; PMH 13, maize hybrid; LM 28, female parent of hybrid PMH 14; LM 29, male parent of hybrid PMH 14; PMH 14, maize hybrid.

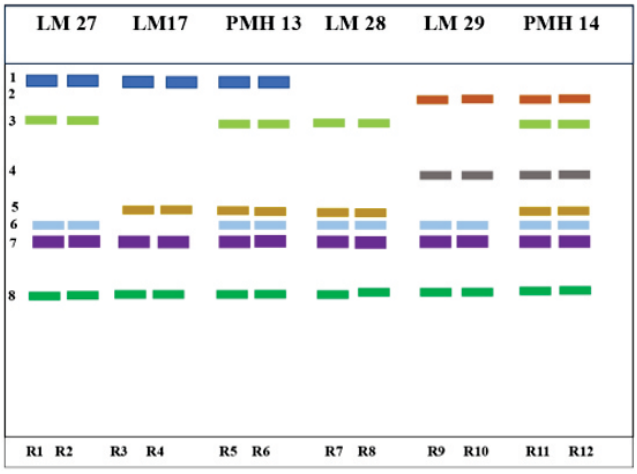


Fig. 3B: Diagrammatic representation of isozyme bands of EST in two maize hybrids and their parents.
LM 27, female parent of hybrid PMH 13; LM 17, male parent of hybrid PMH 13; PMH 13, maize hybrid; LM 28, female parent of hybrid PMH 14; LM 29, male parent of hybrid PMH 14; PMH 14, maize hybrid.

isozyme bands, corresponding to Rm values of 0.400, 0.434, and 0.521, were common to both the maize hybrid PMH 14 and its parents. Four polymorphic bands were identified in hybrid PMH 14: two bands with Rm values of 0.321 and 0.373 shared with the female parent (LM 28), and two bands having Rm values of 0.130 and 0.347, shared with the male parent. Thus, EST zymography can be used to test the hybrid purity of

Table 5: Characterization of EST (esterase) isozyme bands along with Rm value in maize hybrids and their parents

Isoenzyme band	Rm value	LM 27	LM 17	PMH 13	LM 28	LM 29	PMH 14
1	0.113	+	+	+	-	-	-
2	0.130	-	-	-	-	+	+
3	0.321	+	-	+	+	-	+
4	0.347	-	-	-	-	+	+
5	0.373	-	+	+	+	-	+
6	0.400	+	-	+	+	+	+
7	0.434	+	+	+	+	+	+
8	0.521	+	+	+	+	+	+

both maize hybrids PMH 13 and PMH 14 seed lots (Table 5 and Fig. 3A and 3B). Rakshit *et al*., (2011) employed four isozyme markers to distinguish six maize hybrids: one double cross hybrid, two double top cross hybrids and three single cross hybrids. Esterase and alcohol dehydrogenase were found to be the most variable among the four isozymes and can be used to establish identification of maize hybrids.

Profiling of total soluble proteins

Profiling of total soluble proteins using SDS-PAGE exhibited clearly distinct electrophoretic polypeptide banding patterns in maize hybrid PMH 13 and its parents. The analysis of SDS-PAGE detected eight bands in female parent LM 27 with Rm values ranging from 0.456-0.947, twelve bands in male parent LM 17 with Rm value 0.239-0.949 and ten bands in hybrid PMH 13 having Rm values in the range of 0.475-0.97. The molecular weight of polypeptide bands ranged from 20-40 kDa in LM 27; 20-58 kDa in LM 17; and 20-40 kDa in hybrid PMH 13. Three unique polypeptide bands of 58, 50 and 42 kDa corresponding to band numbers 1, 2 and 3 were present only in the male parent LM 17. One polypeptide band of 38 kDa was present in both the male parent and the hybrid. Based on the presence of the latter band, the male parent and the hybrid cannot be distinguished; but the lack of this band is indicative of the female parent. Likewise, the lack of polypeptide bands of 58, 50 and 42 kDa is indicative of the male parent. Seven polypeptide bands of molecular weight 40, 34, 27, 24, 23, 22 and 20 kDa were monomorphic, which were found in hybrid PMH 13 as well as both parents. One polymorphic polypeptide band was present in the hybrid, corresponding to polypeptides of 29 kDa in female (polypeptide band no. 3) and 30 kDa in male (polypeptide band no. 7), which can be used to confirm the hybrid purity of maize hybrid PMH 13. Thus, SDS-PAGE analysis of total soluble proteins can serve as an additional marker system for testing hybrid purity of seed lots by distinguishing true maize hybrids of PMH 13 from its off types (Fig. 4 and Table 6).

The analysis of SDS-PAGE detected ten bands in female parent LM 28 with Rm values ranging from 0.431-0.957, seven bands in male parent LM 29 with Rm values ranging from 0.431-0.966 and eight bands in hybrid PMH 14 with Rm in the range of 0.471-0.975. The molecular weight of polypeptide bands ranged from 20-40 kDa in LM 28; 20-42 kDa in LM 29; and from 20-40 kDa in hybrid PMH 14. Two polypeptide bands of 33 and 26 kDa were present in both the female parent and the hybrid. Based on the absence of these bands, the male parent can be distinguished from the female parent and hybrid. Two polypeptide bands of 29 and 24 kDa were found in the female parent only. Likewise, the presence of a polypeptide band of 42 kDa is an indicator of the male parent. Six protein bands of molecular weight 40, 30, 28, 23, 22 and 20 kDa were monomorphic and were found in hybrid PMH 14 as well as both parents. No

Table 6: Characterization of polypeptide bands observed in SDS-PAGE in hybrid PMH 13 and its parents

Nature of polypeptide band	LM 27 (Female parent)	LM 17 (Male parent)	PMH 13 (Hybrid)	Remarks
		Band No. (MW)		
Monomorphic	1 (40 kDa)	4 (40 kDa)	1 (40 kDa)	Cannot be used for distinguishing parental lines or identification of hybrid purity
	2 (34 kDa)	6 (34 kDa)	3 (34 kDa)	
	4 (27 kDa)	8 (27 kDa)	6 (27 kDa)	
	5 (24 kDa)	9 (24 kDa)	7 (24 kDa)	
	6 (23 kDa)	10 (23 kDa)	8 (23 kDa)	
	7 (22 kDa)	11 (22 kDa)	9 (22 kDa)	
	8 (20 kDa)	12 (20 kDa)	10 (20 kDa)	
Male- and hybrid -specific	_	5 (38 kDa)	2 (38 kDa)	Cannot distinguish male and hybrid.
Male-specific band	_	1 (58 kDa)	_	This is unique band present only in male parent
		2 (50 kDa)		
		3 (42 kDa)		
Polymorphic band	_	7 (30kDa)	4 (30 kDa)	Two protein bands were present in hybrid (band no. 4 and 5) corresponding to two protein bands of molecular weight 29 kDa (band no.3) and 30 kDa (band no.7) in female and male parent, respectively
	3 (29 kDa)	_	5 (29 kDa)	

Table 7: Characterization of polypeptide bands observed in SDS-PAGE in hybrid PMH 14 and its parents

Nature of polypeptide band	LM 28 (Female parent)	LM 29 (Male parent)	PMH 14 (Hybrid)	Remarks
		Band No. (MW)		
Monomorphic	1 (40 kDa) 3 (30 kDa) 5 (28 kDa) 8 (23 kDa) 9 (22 kDa) 10 (20 kDa)	2 (40 kDa) 3 (30 kDa) 4 (28 kDa) 5 (23 kDa) 6 (22 kDa) 7 (20 kDa	1 (40 kDa) 3 (30 kDa) 4 (28 kDa) 6 (23 kDa) 7 (22 kDa) 8 (20 kDa	Cannot be used for distinguishing parental lines or identification of hybrid purity
Female and hybrid specific	2(33kDa) 6 (26 kDa)	_	2(33kDa) 5 (26kDa)	Cannot distinguish female parent and hybrid.
Female specific band	4 (29 kDa) 7 (24 kDa)	_	_	This is unique band present only in female parent
Male specific band	_	1(42kDa)	_	This is unique band present only in male parent

polymorphic band was found. Thus, SDS-PAGE analysis of total soluble proteins cannot be used for testing the hybrid purity of PMH 14 from its off types (Fig. 4 and Table 7).

Vivodik *et al*., (2017) assessed maize genotypes for total seed storage proteins using SDS-PAGE. The procedure produced a total of twenty-three scorable protein bands, though bands that were inconsistent in reproducibility or exhibited occasional variations in sharpness and density were excluded from the analysis. Out of the twenty-three polypeptide bands identified, six bands were found in all accessions and were classified as monomorphic, while seventeen bands were classified as polymorphic. Sharief *et al*., (2005) conducted SDS-PAGE analysis of various maize genotypes, including inbred lines as well as single cross hybrids and a three-way cross hybrid, for their rapid and accurate identification. Gm 2 seeds (inbred lines) had proteins with molecular weights ranging from 69.6 to 149.6 kDa; Sd 63 (inbred lines) proteins ranged from 72.5 to 166.5 kDa; Sd 7 (inbred lines) proteins ranged from 98.7 to 228.1 kDa. SC 10 (single cross hybrid) seeds contained proteins with molecular weights between 121.5 and 281.6 kDa; SC 21 (single cross hybrid) proteins ranged from 73.4 to 259.7 kDa; and TWC 321 (three-way cross hybrid) seeds had proteins ranging from 68.3 to 183.2 kDa. Merza *et al*., (2014) evaluated the total soluble seed proteins using SDS-PAGE in order to differentiate among maize genotypes. Their analysis revealed a total of 118 amplified bands, with a range of 18-86 kDa protein bands. Out of the nine major bands identified, five were polymorphic, and four were monomorphic, resulting in an average polymorphism rate of 55.5 per cent. Similarly, Iqbal *et al*., (2014) used SDS-PAGE to explore variations in the zein percentage of seed storage proteins in maize. The study found variability in the presence and absence, intensity, and molecular size of zein polypeptides. They observed 18 protein bands of proteins, with seven (39%) being monomorphic and eleven (61%) polymorphic, and their molecular weights ranged from 10 to 122 kDa.

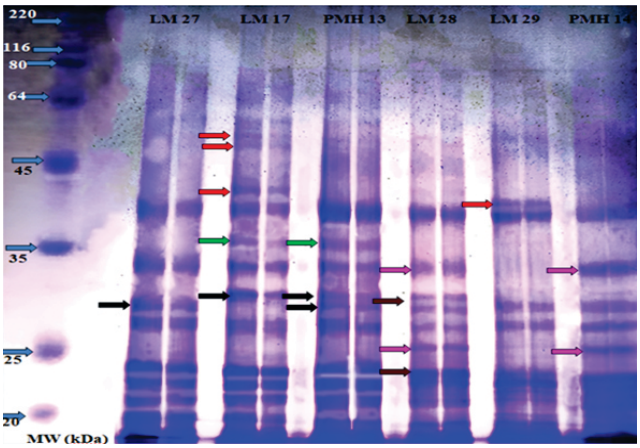


Fig. 4: Protein profiling of two maize hybrids and their parents.
LM 27, female parent of hybrid PMH 13; LM 17, male parent of hybrid PMH 13; PMH 13, maize hybrid; LM 28, female parent of hybrid PMH 14; LM 29, male parent of hybrid PMH 14; PMH 14, maize hybrid.
Green arrow indicates male and hybrid-specific bands; red arrow indicates male-specific bands; black arrow indicates polymorphic bands; pink arrow indicates female and hybrid-specific bands; and brown arrow indicates female-specific bands.

Conclusion

The study was conducted with the objective of identifying physiological and biochemical markers at the seedling stage for hybrid purity testing of maize hybrids PMH 13 and PMH 14. Both the hybrids PMH 13 and PMH 14 had higher speed of germination, seedling length and dry weight, SVI I and II, with a concomitantly

shorter mean germination time as compared to their respective parental lines. Zymograms of SOD and EST can be employed to test PMH 13 and PMH 14 hybrid purity, while the POD zymogram can be utilized to detect selfed female seed in PMH 14 seed lots. SDS-PAGE analysis of hybrid PMH 13 revealed one polymorphic polypeptide band corresponding to a polypeptide of 29 kDa in female and 30 kDa in male, which can be used for confirming the hybridity of maize hybrid PMH 13; while hybrid PMH 14 did not reveal any polymorphism in polypeptide bands.

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