Effects of spring and autumn seasons on the variability among sunflower (*Helianthus annuus* L.) accessions for pollen viability, germination and morphology

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ABSTRACT: In general exclamation pollen is not competent to maintain the viability and germination for longer time. High temperature has great adverse effects on pollen viability and ultimately it lessens its fertilization ability. In Pakistan there are two sunflower sowing seasons i.e. spring and autumn, having different climatic conditions. The research was conducted in the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Ten sunflower accessions developed and maintained by the Oilseed Research Group, Department of Plant Breeding and Genetics, were evaluated for pollen viability, germination and morphology in spring and autumn seasons. Split plot design with three replications was used for this study. All the accessions, seasons and their interaction showed non-significant difference for pollen viability and highly significant difference for pollen germination. The accession G-33 and G-7 had better performance for both pollen viability and germination under spring and autumn seasons respectively. Autumn season showed higher percentage for pollen viability and germination than spring season. A high percentage of pollen viability and germination helps in seed setting and this may be useful in hybridization, artificial breeding and to study the sterility problems. These selected accessions may be used in breeding for the improvement of economically important achene yield trait.

Key Words: Sunflower; Spring and autumn seasons; Pollen viability; Pollen germination; Pollen morphology

1. INTRODUCTION

Sunflower is the second most important crop worldwide for oil production. Presence of vitamin A, B complex, D, E, K, calcium and phosphorous [1] in its oil makes healthy cooking oil [2]. Its oil is premium due to light colour, good in taste, high smoke point, oxidative stability, good dietary qualities and high level of unsaturated fatty acids. Sunflower crop is not bound to the season, fits well in the cropping pattern of Pakistan and is grown in spring and autumn seasons. But its achene yield per hectare (1345 kg/ha) is far less than other countries like Turkey (2036.0 kg/ ha), China (1752.6 kg/ha) and USA (1567.1 kg/ha) [3].

Pollen study is required to determine the genetic behaviour, because pollen is the source of genetic variation. As it is the main vector which inserts all the genetic material in the next generation [4]. Pollen grains comprise a hard coat that guards the sperm cells during their transfer from the stamens to the pistil of flowering plants. After pollen grain transfer on a compatible pistil, germination and production of pollen tube starts which transfers the sperm.

Climate has great effect on the pollen viability and germination in different crops [5]. Pollen grain is prone to heat and humidity in *Triticum aestivum* L. [6], *Sorghum bicolor* L. [7] and *Hordeum vulgare* L. [8]. Unfortunately very little information is reported about the interaction of climate and pollen production in sunflower [9, 10] and between pollen viability and yield in sunflower [11]. Sunflower pollen viability is highly influenced by the climate variation particularly by temperature [12]. Study of pollen production provides the significant knowledge for breeding [13].

The study of pollen is very useful in the breeding programs for artificial pollination procedures [5], creation of sterile lines, hybridization [14] and in the evolutionary ecology [15]. Viable pollen affects the achene yield because only these can fertilize and germinate. A very low rate of viable pollen that loads on stigma to fertilize the ovules can alter the anatomy and development of achene causing reduction in yield [16, 17, 18, 19, 20, 21, 22].

Pollen viability is an important biological trait and is tested by using different stains or by demonstration of germination percentage. Tetrazolium based stains are also used for the pollen viability test [23]. Colourless

tetrazolium is reduced to coloured formazan by the oxidative capacity of the pollen grains [24] and viable pollen can be observed by intensity of colour.

Keeping in view the situation current study was carried out with the objective to determine the genetic variability among sunflower accessions for pollen morphology, viability and germination percentage under spring and autumn seasons.

2. MATERIAL AND METHODS

2.1 Experimental Condition

The research work was conducted in the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan during 2011. Faisalabad is situated in the rolling flat plains of North East Punjab. It is between longitudes 73˚-06́ East, latitude 30˚-26́ North and altitude is 184.4 m, possesses arid climate and loamy soil in field. Average minimum and maximum temperature of spring and autumn crop season in 2011 is presented in Fig. 1 and relative humidity and total rainfall in Fig. 2.

Fig.1 Monthly average maximum and minimum temperatures during spring and autumn crop season of 2011

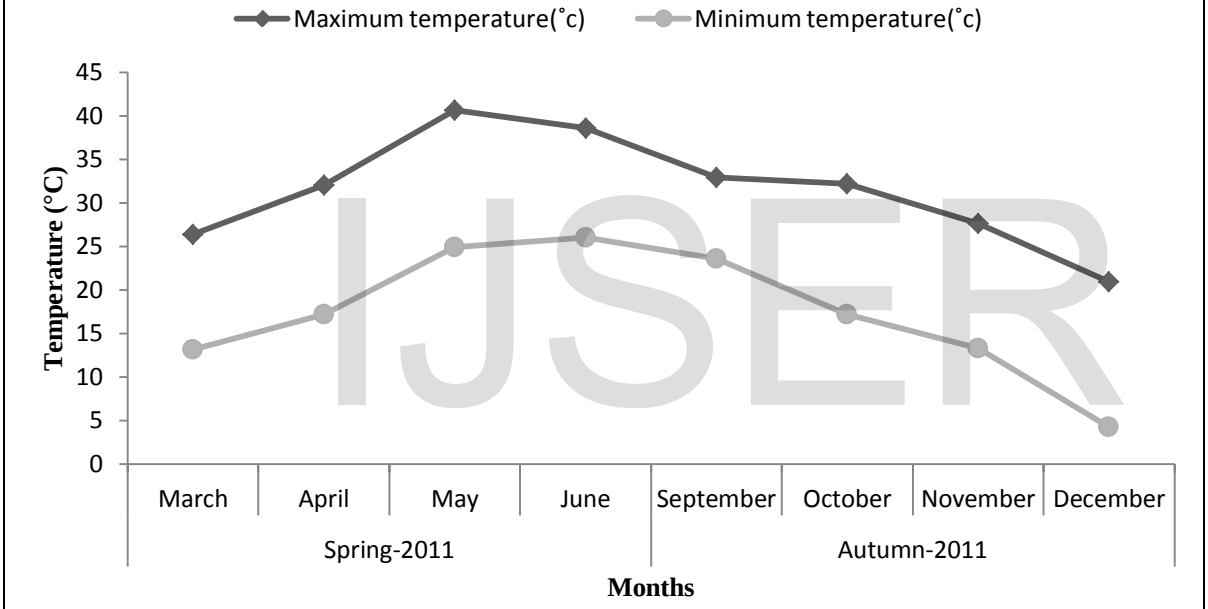
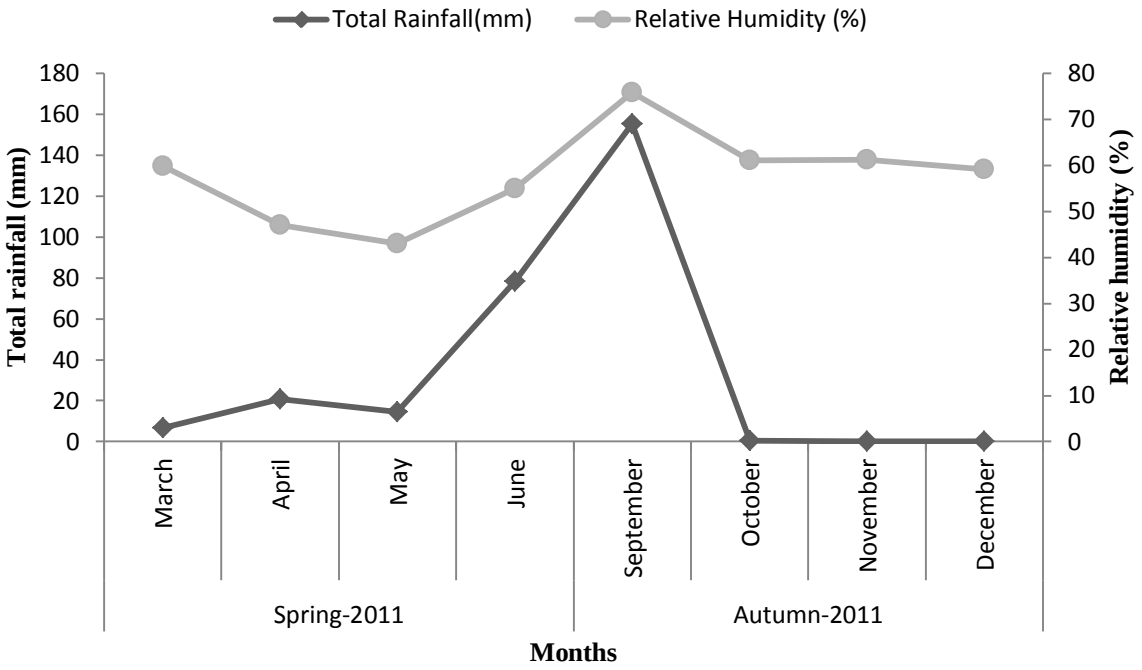


Fig. 2 Monthly average relative humidity (%) and total rainfall (mm) spring and autumn crop season of 2011



2.2 Experimental Material

The experimental material compromised of 10 sunflower accessions viz. HBRS-1, A-48, G-33, G-56, A-23, A-30, A-45, G-8, G-40 and G-7. The accessions were developed and maintained by the Oilseed Research Group, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan.

2.3 Experimental Layout

This experiment was laid out under split plot design with three replications during the 2011 spring and autumn seasons. Crop was grown in spring season on 14 March, 2011 and in autumn season on 8 September, 2011. Row to row and plant to plant distance was maintained 75 cm and 25 cm, respectively.

2.4 Methods

Fresh pollen grains were collected in petri dish from single plant of each accession per replication. Pollen grains were observed for following parameters.

2.4(a) Pollen viability test

Fresh pollen grains were taken on glass slide in a drop of 0.5% 2, 3, 5 Triphenyl Tetrazolium Chloride (TTC) in the 15% sucrose solution. Sample was covered with cover slip immediately to avoid the oxidation and was placed in incubator for 2 hours [25]. After this treatment viability was observed by identifying viable pollen (reddish colour) and non-viable (light brown) under microscope (Olympus 1×50).

2.4(b) Pollen germination

For pollen germination fresh pollen grains were collected and kept in petri dish. A 15% sucrose solution was prepared in a mixture of 50% H_3BO_3 (2×10^{-3}M) and 50% $Ca(NO_3)_2$ (6×10^{3}M) by volume. Pollen grain sample was taken on glass slide in a drop of sucrose solution and covered to avoid oxidation. This slide was kept in a petri dish lined with wet filter paper and was kept in incubator for 2 hours [25]. The slides were then observed under microscope (Olympus 1×50), pollen grains germinating pollen tubes were counted and percentage was calculated.

2.4(c) Pollen Morphology

The pollen grains of the accessions under study were also observed for pollen shape, colour and appearance by using microscope (Olympus 1×50).

2.5 Statistical analysis

The data were analyzed statistically following analysis of variance technique [26] to determine the differences among accessions, seasons and their interaction for pollen viability and germination. Tukey's test [27] was used to find the differences among accessions within and between the seasons.

3. Results and Discussion

3.1 Pollen viability

Viable and non-viable pollen grains were differentiated by colour, viable pollen with reddish colour and non-viable pollen with light brown colour (Fig. 6). Analysis of variance showed that accessions, seasons and their interaction had non-significant differences (Table 1) for this trait. Pollen viability ranged from 72% to 87% in spring season while 73% to 89% in autumn. Pollen viability has been reported from 53.54% to 90.97% in literature [28, 29, 30, 31, 32, 33, 34]. Pollen viability range in our breeding material is comparatively more than the range found in literature. All accessions had more than 75% viable pollen except A-23 and G-56 in spring season and A-30 and HBRS-1 in autumn (Fig. 3). The accession G-33 followed by A-45 and A-30 showed the highest pollen viability percentage under spring season, while accession A-48 followed by G-8 and G-7 in autumn season. Although difference between the growing seasons for viability of pollen grains was non-significant yet, pollen viability percentage was slightly higher in autumn season than spring (Fig. 5).

Table I: Analysis of variance for pollen viability and germination in sunflower accessions

Sources of variation	Pollen viability	Pollen germination
Replication	0.22	104.4
Season	2.82	3450.4**
Error 1	69.6	23.12
Accessions	80.7	820.9**
Season x Accessions	117.3	1235.45**
Error 2	116.1	135.2

** = Highly significant ($P<0.01$)

3.2 Pollen germination

Pollen germination was observed by formation of pollen tube (Fig. 7). Only viable pollen grains which are properly transferred on the stigma may germinate pollen tube in the pistil and fertilize the ovules (Connor and Hall, 1997). Accessions, seasons and their interaction showed significant differences for pollen germination (Table I). It indicates that seasons had strong effects on the germination of accessions. Pollen germination ranged from 14% to 57% in spring season whereas 17% to 71% in autumn (Figure 2(b)). Pollen germination range in literature is 40.40% to 74.6% [31, 32, 35]. Pollen germination of breeding material studied in this experiment is comparable with the range found in literature. Accessions showed more than 20% pollen germination except G-56, A-30 and A-45 in spring season. In autumn season all the accessions had more than 35% pollen germination except A-45 and G-33. The accession A-23 followed by G-8 and G-33 showed maximum pollen germination (57%) in spring season and it was significantly different from all accessions except A-48, G-33 and G-8. While G-7 followed by A-30 and HBRS-1 showed highest pollen germination (71.67%) in autumn season and it was non-significantly different from all accessions except A-48, G-33 and A-45. On the whole pollen germination percentage was higher during autumn growing season compared to that in spring season because in Pakistan the temperature at the time of flowering and pollination during spring is usually higher than that in autumn season which may retard the pollen tube germination [12].

Fig. 3 Pollen viability of sunflower accessions in spring and autumn crop season

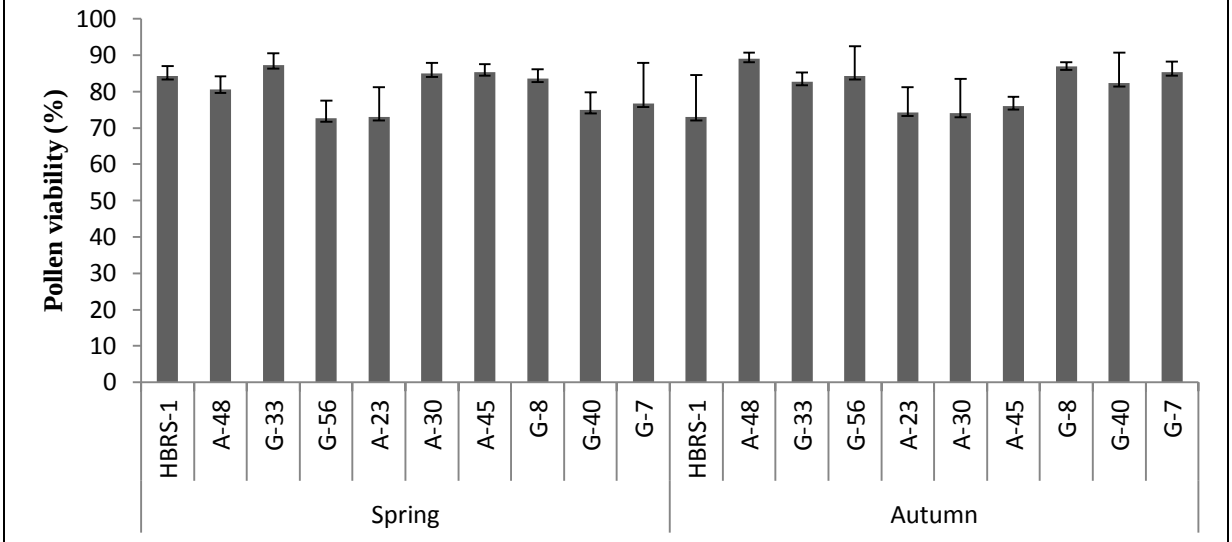


Fig. 4: Pollen germination of sunflower accessions in spring and autumn

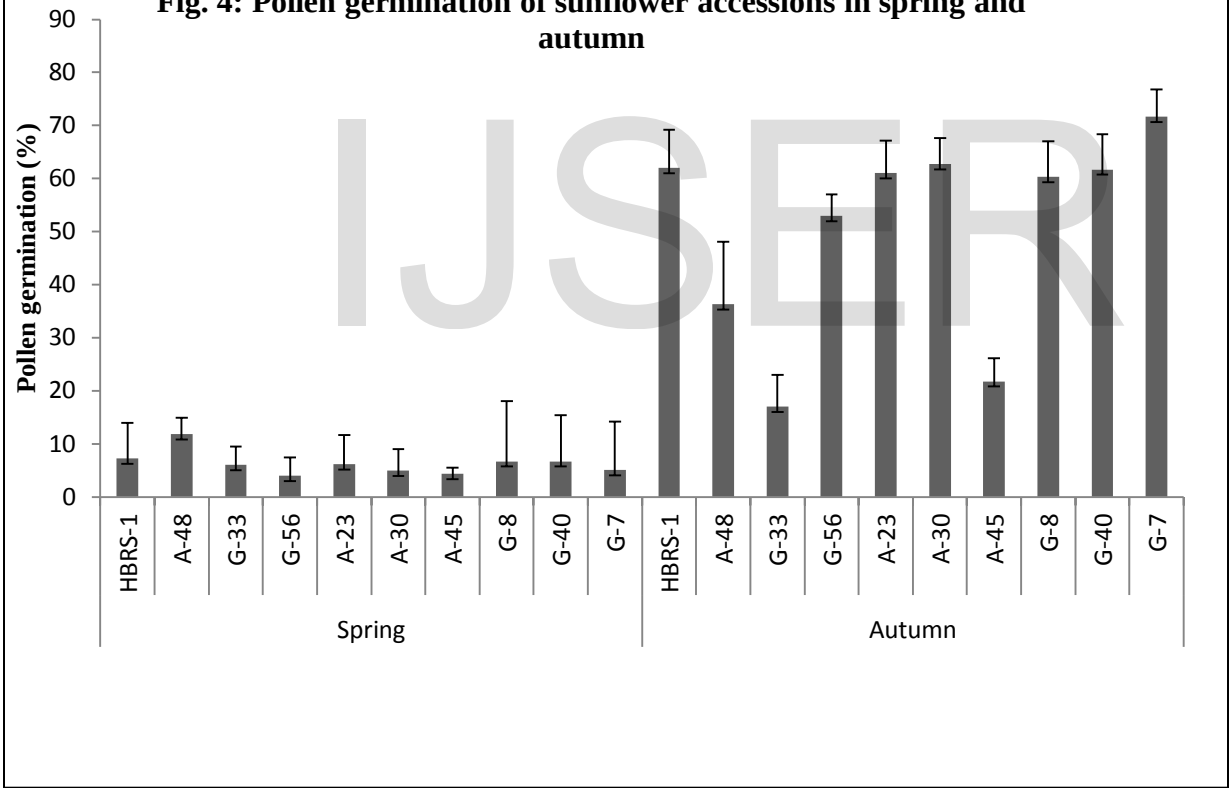
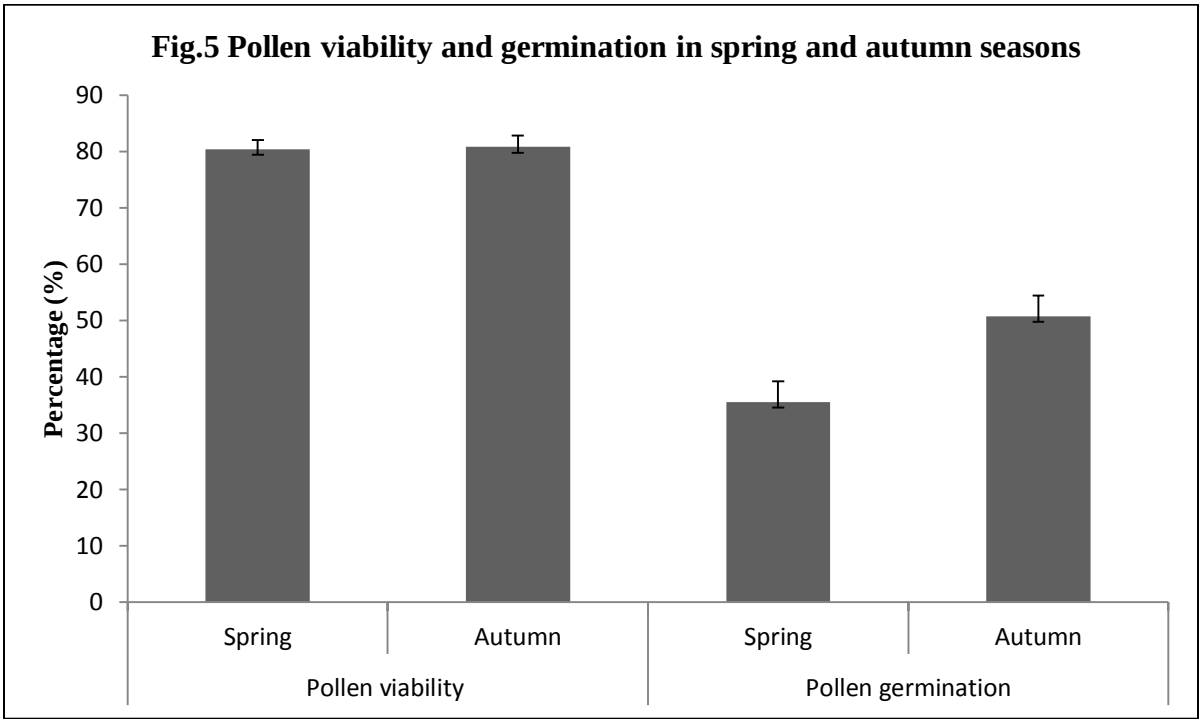


Fig.5 Pollen viability and germination in spring and autumn seasons



3.3 Pollen morphology

Samples of pollen grains were examined for the pollen shape, colour and appearance under digital microscope (Olympus 1×50) (Table II). All the accessions had pale yellow colour in autumn season. While in spring season all the accession had pale yellow except G-8, A-30 and G-40 (yellow) (Fig. 8). Golf ball shape and pines pollen appearance were observed for all accessions in both seasons (Fig. 9).

Table II Different pollen characters of studied sunflower accession

Seasons	Accession	Pollen colour	Pollen shape	Pollen appearance
Spring	HBRS-1	Pale yellow	Golf ball	Pines
	A-48	Pale yellow	Golf ball	Pines
	G-33	Pale yellow	Golf ball	Pines
	G-56	Pale yellow	Golf ball	Pines
	A-23	Pale yellow	Golf ball	Pines
	A-30	Yellow	Golf ball	Pines
	A-75	Pale yellow	Golf ball	Pines
	G-8	Yellow	Golf ball	Pines
	G-40	Yellow	Golf ball	Pines
	G-7	Pale yellow	Golf ball	Pines
Autumn	HBRS-1	Pale yellow	Golf ball	Pines
	A-48	Pale yellow	Golf ball	Pines
	G-33	Pale yellow	Golf ball	Pines
	G-56	Pale yellow	Golf ball	Pines
	A-23	Pale yellow	Golf ball	Pines
	A-30	Pale yellow	Golf ball	Pines
	A-75	Pale yellow	Golf ball	Pines
	G-8	Pale yellow	Golf ball	Pines
	G-40	Pale yellow	Golf ball	Pines
	G-7	Pale yellow	Golf ball	Pines

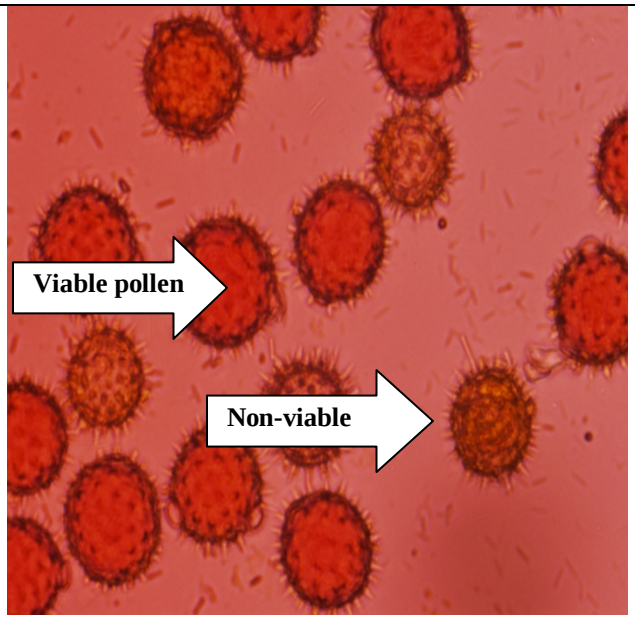


Fig. 6: Pollen viability reddish colour (viable pollen) and light brown (non-viable pollen) in sunflower accessions

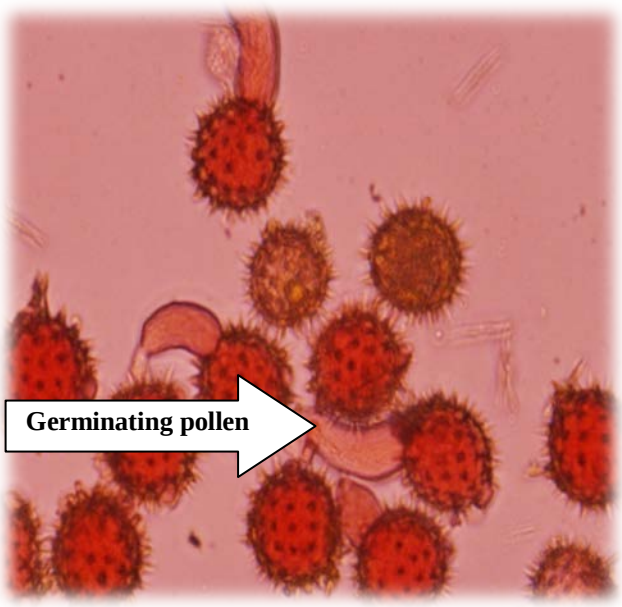


Fig. 7: Pollen germination in sunflower accessions

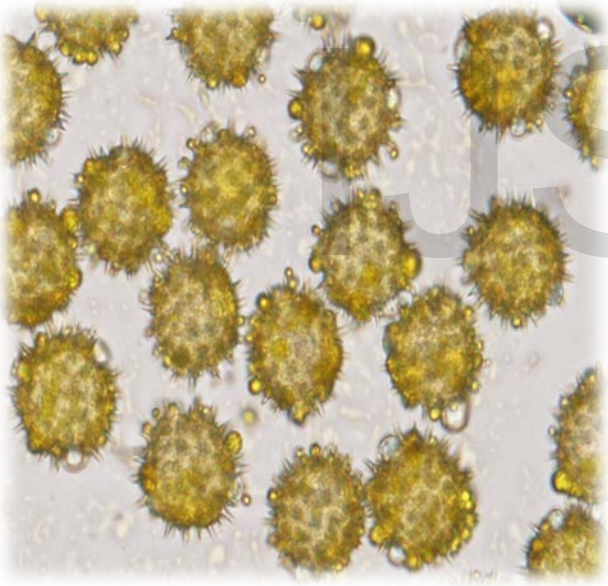


Fig. 8: Pollen colour of sunflower accessions

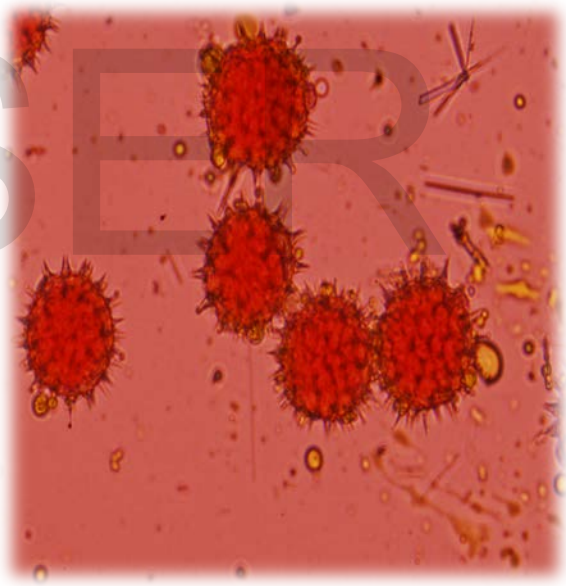


Fig. 9: Pollen shape and appearance of sunflower accessions

4. Conclusion

Presence of the genetic differences among the accessions for pollen germination in different growing seasons may be exploited in the hybridization program and also in artificial breeding. Performance of sunflower accessions in both seasons for pollen viability and germination suggests their use in the future to create sterile lines, evolutionary ecology and also in seed setting. The accessions G-33 and G-7 showed better performance for pollen viability and germination under spring and autumn season respectively. On the whole accessions had better performance in autumn season than spring, it is may be due to high temperature at the flowering and pollination in spring season in Pakistan which may abort the pollen grain and causing low pollen germination.

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