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Phenological and Floral Variations in *Moringa oleifera* Ecotypes for Reproductive Adaptations in Subtropical Conditions

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Abstract: *Moringa oleifera* is an important medicinal tree valued for its highly nutritious pods. It is widely distributed in tropics and native to foothills of Himalayas. The present study aimed to assess the phenological and floral studies of landraces and high-yielding ecotypes of *Moringa oleifera* procured form diverse agro-ecological zones of Indian subcontinent. The observations revealed significant variation in timing of occurrence of different phenophases in these ecotypes. The ecotypes of subtropical origin initiated vegetative and floral bud events relatively earlier than those from tropical, arid and semi-arid regions. Ecotype S1 (*Bhagya*) was found potentially viable ensuring pollination and post fertilization success due to long petal size and less filament length. Higher pollen viability and *in-vitro* germination with more pollen size was observed in S7 ecotypes, which could be exploited for future breeding programmes. Differences were found non-significant for days taken to flower anthesis, anthers dehiscence, pollen shape and visitation of pollination vectors noticed during flowering. The study revealed greater climate resilience and reproductive success in ecotypes of *M. oleifera* by their unique floral and pollen traits. In addition, the ecotypes exhibited variation in growth and morphological characteristics that are deemed as potentially beneficial silvicultural traits for adoption in agroforestry systems.

Keywords: *Moringa oleifera,* Ecotype, Phenology, Floral biology, Reproductive adaptation

Moringa oleifera Lam., is a member of the monogeneric family *Moringaceae* and genus has 13 species, of which *M. oleifera* is widely cultivated in tropical and subtropical world. *Moringa oleifera* is considered to have its origin in foothills of Himalayan region (Lalas et al 2012) but nowadays distributed in Asia, the Arabian peninsula, East and West Africa, and some part of American countries (Mishra et al 2011) owing to its rapid growth, drought tolerance, and wider adaptability. It grows best in temperatures ranging from 25°C to 35°C (Thurber and Fahey 2009). Flowering period varies widely depending on the variety and the location and usually flowers once a year but can flower twice in more tropical part of the world. In regions with no change of temperature or precipitation throughout the year, flowering may be more or less continuous. In India, the flowering occurs between March to May, in Moreno, Buenos Aires Province, Argentina, the flowering period is between November and March but some flower were differentiated on plants grown in greenhouse during June (Thiberge 2014). For effective production of immature fruits and seeds, appropriate pollination and fertilization techniques are required which depends upon flower dimensions and pollen characteristics. Extremely low fruit low fruit set rates, *i.e.* 1.5% in dry and 0.31% in the rainy season have been reported as compare to its abundant floral display; the reason for its less fruit set are still unknown (Krieg et al 2017). The slight change in environment conditions leads to the disruption of flower morphology followed by pollen viability and stigma receptivity, fruit setting and pod percentage (Radice and Giordani 2018). The drumstick tree is a mixed mating species adapted for out-crossing, although it is also possible to self-cross (Muluvi et al 2004). Keeping in view of above facts and discrepancies, the present study had been planned to acquire the detailed knowledge about its phenological behaviour, flower morphology and floral studies of among landraces and cultivated varieties comparing with the sub-tropical or regional ecotypes of *Moringa oleifera* for floral adaptation in subtropical conditions of north western India.

MATERIAL AND METHODS

Climate and plant material: The experiment was conducted at Punjab Agricultural University, Ludhiana. The experimental site is located at an elevation of 247 m amsl (30°54´N latitude and 75°48´E longitude). The area falls in the central plain agro-climatic zone of Punjab. The region is categorized by sub-tropical environment. In May to June and in December to January, the region has hot summer and severe winter. The coldest temperature might fall up to 4°C or even below, and in the summer time the maximum temperature might rise to more than 46°C. It is not usual to get frost. The land is deep, well drained, granular, low humus textured loam. The soil's pH is neutral. The average yearly rainfall is 760 mm and during June and September around three-fourth of this year's south-west monsoon. The meteorological data during the study period (2020 to 2021) has been depicted in Figure 1 and 2. In the present study, eight ecotypes were used for phenological and reproductive biology studies. Seed of four promising varieties adopted at national level and three local germplasm of *Moringa oleifera* were procured from different states of India, and one wild ecotype (S4) maintained at PAU, Ludhiana was used as check source (Table 1). The evaluation trial was established since August 2017 at spacing of 3.5×2.0 m in three replications with plot size of 5 plants/treatment following complete randomized block design.

Field and laboratory observations: Plants were pollarded at 6 feet height to avoid the breaking damage due to having very soft wood. Phenological observations like growth habit, leaf retention period, functional type (Singh and Kushwaha 2005), vegetative bud swell and burst; and reproductive traits like floral bud swell and burst, flowering span and days to anthesis were taken on five branches selected on three trees with three replications. Bud characteristics were measured for two consecutive years, 2019-20 and 2020-21, while remaining phenological and reproductive traits were recorded only in 2021. Flower morphological characteristics, *i.e.* number of flowers per panicle, peduncle and pedicle length (cm), flower length and width (cm), sepal length (mm), petal length (cm), filament and style length (cm) were recorded on 15 flowers per replication. The mode of anther dehiscence was carefully observed and recorded for seven seed source, namely, S1, S2, S3, S4, S7, S9 and S10. For this observation, flowers from each seed source was taken and observed from floral bud burst to complete development of flower and time of anther dehiscence was recorded. The activities of flower visitors/pollinators were carefully watched and identified with the help of the Department of Entomology, PAU, Ludhiana, throughout the flowering period.

Pollen and stigma studies: Pollen viability was tested using 2% acetocarmine dye. The ocular micrometre was standardised with the stage micrometre to analyse pollen morphology. Under the electron microscope, the shape/form of the pollen was studied. The *in vitro* germination of pollen extracted from different sources was tested immediately after collection. Freshly collected pollen were tested to check it's *in vitro* germination using five different growing media, *viz*., 5, 10, 15 and 20% sucrose and 200 microgram/ml boric acid for all sucrose combinations along with distilled water as control treatment. Stigma receptivity was visually observed of stigmatic surface. From 24 hours before the opening of flower till it wilted completely, the stigma's appearance was continuously observed. The stigma was considered receptive when stigma exude white watery substance and the colour of stigma changes from greenish white to pinkish white, whereas dark brown or black coloured of stigma was accounted non-receptive.

Statistical analysis: The mean data recorded on various observations (phenological and reproductive parameters) were subjected to statistical analysis with the help of SPSS *version* 21 software.

RESULTS AND DISCUSSION

Phenological behaviour: Phenological events are the result of internal factors such as biorhythms, which are regulated by the genetic constitution of the species and environmental factors (Orlandi et al 2007). The significant differences were observed for the time of occurrence of phenological events which might be due to the wide variations in local environmental conditions (Fig. 1, 2). Ecotypes S7, S9, S1 and S2 are evergreen in their respective original habitat while their functional type was found to change in subtropical conditions (Table 1, 2). All ecotypes showed a high vegetative seasonality showing three patterns in functional type, three types of growth habit. The leaves of the south Indian ecotypes showed burnt symptoms in January due to chilling temperature and remain leafless till mid-March, while local source (S4) does not have any leaf burning symptoms during winter months. Their leafless nature may be varying with original locality but the changes in their functional type are due to G×E interactions (Singh and Kushwaha 2005, Sauvadet et al 2021).

The significant differences were observed in mean number of days taken for vegetative growth, bud characteristics, and days to anthesis among the eight ecotypes (Table 3). Vegetative bud growth was concentrated from 2nd fortnight February to 1st fortnight of November. Ecotype S11 was first to be noticed for swelling its vegetative buds in 3rd week of February in 2020, while S4 was first noticed in 2nd week of February in 2021 which was almost 17 days week earlier than 2020 (Fig. 3) as it is adapted to prevailing environmental conditions. However, bud initiation was observed only 2-6 days earlier in procured ecotypes under subtropical climate. The minimum number of days took from vegetative bud swelling to bud bursting was observed in subtropical local source (11 and 15 mean days) for both years, while maximum (25 and 24 mean days) were observed in semi-arid sources S1 and S11 followed by tropical sources, namely, S7 (24 days) and S2 (22 days) (Fig. 4). Vegetative bud swelling was advanced by at least 3 days to 17 days in 2021 than 2020. This could be due to the mean maximum temperature in 2021 was higher in February and very less precipitation occurred in winter months of 2020. The buds began to break early due to an early increase in threshold temperature (the temperature required for swelling and bursting). The differences in time of occurrence of vegetative bud swelling and bursting between 2020 and 2021 was very less in majority of ecotypes, which reflects that the ecotypes are adapted under subtropical conditions (Fig. 3, 4). More precipitation in September and October 2021, vegetative bud swelling and bud bursting span was found to be increased by 4 and 6 mean days in *Moringa* ecotypes, respectively. The increase in temperature affects more S4 for vegetative bud swell and S7 for bud burst span in 2021. The findings accord with Orlandi et al (2007) in *S. Acutifolia*.

The change from vegetative to reproductive growth is triggered by environmental variables. Mean flowering peak was seasonal and genotypically controlled, with similar flowering patterns for both years, and differences in date of occurrence of the phenophases among ecotypes (Table 3). The floral bud swelling comparatively delayed (2nd fortnight of March 2020) in subtropical ecotype (S4) which is closely followed by S9 tropical ecotype (Fig. 5). However, it was observed 30 mean days (2nd fortnight of February 2021) earlier in S4 during environmental conditions of 2021. Ecotypes S1, S2, S7 and S9 showed floral bud swelling two times in 2020 and 2021 as they showed flowering twice in a year. The time taken from floral bud swell to bud burst was minimum in S1 semi-arid ecotype (11 mean days) during 2020 and 2021. However, maximum was in S4 (18 mean days) (Fig. 6). The significant differences were observed for duration of floral bud swelling span which was continued for 49.75 and 45.75 mean days in 2020 and 2021, respectively (Table 3). The bud bursting span was 51.75 and 48.50 mean days in 2020 and 2021, respectively. Thus, the floral bud swelling and bud bursting span was found increased by 2 and 3 mean days in 2021. Data pertains to flower anthesis revealed that maximum anthesis period was observed in S2 and S7 (26 mean numbers of days), while minimum was noticed in S11 (23.00 mean days).

The change in weather conditions has less effect on

Table 1. Details of *Moringa oleifera* ecotypes procured from geographical origin

Agro-climatic zone	Code	Identity	Ecotype (CL/LR)	Geographical origin	Latitude (°N)	Longitude (°N)	Altitude (m)
Tropical region	S9	*ODC-3*	Cultivar	Indian Agri Farm, Tamil Nadu	8.15	77.59	31
	S7	*PKM-1*	Cultivar	Tamil Nadu Agricultural University, Tamil Nadu	11.31	76.93	310
	S2	*Konkan Ruchira*	Cultivar	Dr. Balasaheb Sawant Konkan Krishi Vidypeeth, Dapoli, Maharastra	17.75	73.18	164
Subtropical region	S4 (Check)	PAU-1	Landrace	Ludhiana, Punjab	30.90	75.81	247
Semi-arid region	S1	*Bhagya*	Cultivar	University of Horticulture Science, Bagalkot, Karnataka	16.18	75.69	542
	S11	Mandya local	Landrace	Mandya, Karnataka	12.52	76.89	683
Arid region	S3	Dantiwara local	Landrace	Sardar Krushinagar Dantiwada Agricultural University, Gujarat	25.24	73.32	311
	S10	CAZRI, local	Landrace	ICAR-CAZRI, Jodhpur, Rajasthan	26.25	72.99	236

Note: CL – Cultivar; LR – Landrace

Table 2. Start and end dates of leaf retention and leafless phenophase in *Moringa oleifera* ecotypes

Moringa ecotypes	Growth habit	Leaf retention period		Leaf less period		Functional type
		Start	End	Start	End	
S9	Compact	20-Mar-20	10-Jan-21	11-Jan-21	11-Mar-21	Fully deciduous
S7	Compact	18-Mar-20	15-Jan-21	16-Jan-21	10-Mar-21	Fully deciduous
S2	Compact	Throughout year		No		Semi-deciduous
S4	Erect	Throughout year		No		Evergreen
S1	Compact	21-Mar-20	10-Jan-21	11-Jan-21	12-Mar-21	Fully deciduous
S11	Intermediate	16-Mar-20	18-Jan-21	19-Jan-21	14-Mar-21	Fully deciduous
S3	Intermediate	Throughout year		No		Semi-deciduous
S10	Intermediate	Throughout year		No		Semi-evergreen

regional ecotypes than local one and might be due to their rigid phenological behaviour. Floral bus set time was observed earlier in 2021 (Fig. 5, 6) but bud swell and burst span was found to be increased with increasing mean temperature (Table 3). Time taken for bud bursting was found decreased due to increase in temperature in 2021. Thus, increasing temperature may be favourable for floral adaptation in procured ecotypes. The minimum effect of increasing temperature in 2021 was observed in S11 ecotype (3 mean days earlier). It was due 2°C-5°C more day and ~2°C more night temperature in February and March 2021 (Fig. 1 & 2). Variation in floral bud swelling and bursting are in agreement with finding of Radhamani et al (1993) on reproductive biology and breeding system studies in *Tamarindus indica*. Similar trends of findings were reported by Pant et al (2003) on *Grewia optiva,* Wani (2005) on *Bauhinia variegata* and Chauhan et al (2009) on *Dalbergia sissoo*. The delay in bud swelling and its bursting may be attributed to the climatic factors as such as temperature and rainfall. During 2020 the occurrence of comparatively cooler

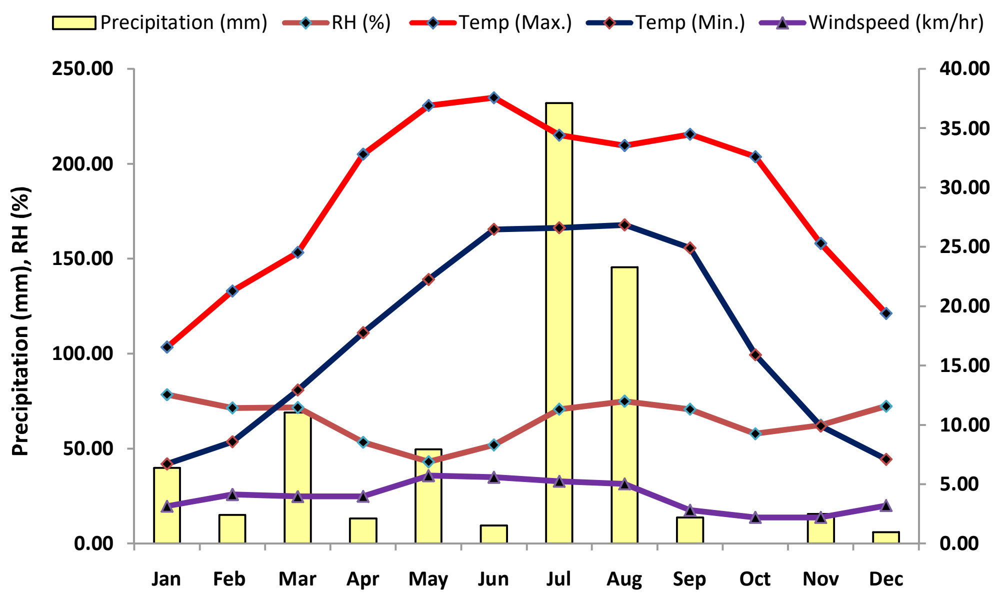

Fig. 1. Mean monthly meteorological parameters of experimental site during 2020

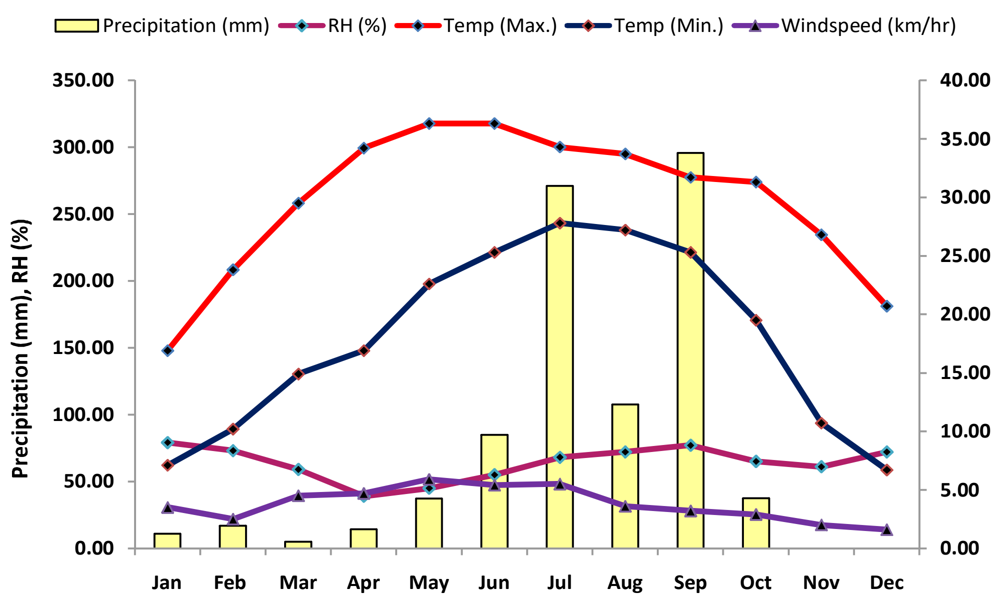

Fig. 2. Mean monthly meteorological parameters of experimental site during 2021

winter along with less precipitation may have delayed the phenological events.

Flower morphology: The ecotypes showed significant differences in the floral morphometric characteristics (Table 4). Maximum number of flowers per panicle was observed in S7 (93 flowers per panicle) and all ecotype showed higher flowers per panicle than local ecotype (78 flowers per panicle) with the average of 85.38 flowers per panicle. The number of flowers was higher in regional ecotypes than the check ecotype with the mean value of number of flowers per panicle (85.38 flowers per panicle) and similar with the studies of Raja et al. (2013) in *M. oleifera* grown in semiarid and arid ecosystem. The average length of peduncle and pedicel were 1.55 and 1.83 cm, respectively. Ecotype S7 (1.99 cm) had more peduncle length than the check ecotype S4 (1.81 cm), which is preferable to avoid the space competition with simultaneously occurred vegetative flush growth as described by Singh et al. (2021). Less pedicel length is best to reduce the flower drop as observed in majority of ecotypes except S10 and S7 ecotype. Flower width was found higher in almost all ecotypes than check source which is directly correlated with the petal length and is preferred for pollination visitors to provide the sitting space for pollinator insect. Filament length was almost lesser than the check source and no specific pattern was for the style length. Thus,

Table 3. Variation in mean number of days for different phenophases in *Moringa oleifera* ecotypes and their flowering span during the years 2020 and 2021

Moringa ecotypes	Leaf retention period	Leaf less period	Vegetative bud swell span		Vegetative bud burst span		Floral bud swell		Floral bud burst		Days to anthesis 2021	Flowering span	
			2020	2021	2020	2021	2020	2021	2020	2021		2020	2021
S9	296	59	252	253	237	247	48	57	43	54	25.00	April-May & Aug-Oct	April-May & Sep-Oct
S7	303	53	248	257	235	248	50	52	46	47	26.00	April-May & Oct-Dec	April-May & Oct-Nov
S2	365	0	234	235	225	231	52	51	49	49	26.00	April-May & Sep-Oct	April-May & Aug-Oct
S4	365	0	227	239	226	235	47	54	39	52	25.00	April-May	March-May
S1	295	60	254	253	240	244	49	55	48	52	24.00	April-May & Sep-Oct	March-May & Aug-Oct
S11	308	54	246	256	236	242	51	46	47	42	23.00	April-May	April-May
S3	365	0	250	244	238	234	50	55	48	52	25.67	April-May	April-May
S10	365	0	240	245	232	239	51	44	46	40	24.33	April-May	April-May
Mean	332.75	28.25	243.88	247.75	233.63	240.00	49.75	51.75	45.75	48.50	24.88	-	-
CD (p=0.05)		-	6.57	5.52	5.94	5.37	4.36	4.04	4.84	4.42	4.27	-	-

Table 4. Vegetative and reproductive morphometric variability among 8 ecotypes of *Moringa oleifera*

Moringa ecotypes	Number of flowers per panicle	Peduncle length (cm)	Pedicel length (cm)	Flower length (cm)	Flower width (cm)	Sepal length (mm)	Petal length (cm)	Filament length (cm)	Style length (cm)
S9	83.00	1.20	1.69	1.78	2.47	2.38	1.39	0.42	0.29
S7	93.00	1.99	2.02	2.07	2.85	3.05	1.63	0.43	0.28
S2	87.00	1.47	1.81	1.84	2.56	2.57	1.37	0.45	0.36
S4	78.00	1.81	1.92	1.85	2.43	2.79	1.41	0.53	0.30
S1	85.33	1.51	1.53	1.96	2.45	2.95	1.69	0.40	0.33
S11	84.33	1.81	1.63	1.71	2.49	2.57	1.30	0.40	0.29
S3	90.67	1.25	1.92	1.83	2.62	2.68	1.42	0.41	0.33
S10	81.67	1.32	2.08	1.89	2.43	2.49	1.50	0.54	0.29
Mean	85.38	1.55	1.83	1.87	2.54	2.69	1.46	0.45	0.31
CD (p=0.05)	8.05	0.66	0.35	0.15	0.28	0.05	0.14	0.05	0.03

ecotype S1 was observed best among *M. oleifera* seed sources due to having more petal length and less difference between filament and style length which is desired for optimum pollination and higher rate of fruit setting. Thus, we found that geographical origin and prevailing environmental factors does not have any significant effect on flower morphology and dimensions among ecotypes. However, the ecotypes originated in the different habitats but did not have more difference in their floral characteristics and dimensions as compare to local ecotype. This differences between ecotypes are not expected as ecotypes of the same species (*Moringa oleifera*) would have similar floral characters because ecotypes are more ecologically similar (Burns and Strauss 2011). But the time of occurrence of phonologically events have changed due to G×E interaction.

Pollen and stigma: Flower anthesis is of a forenoon pattern (6.00 hrs to 13.30 hrs) after which pollen anthesis and nectar secretion take place (Table 5). The maximum dehiscence of anthers took place between 6:00 am to 12:00 pm, of which peak dehiscence was observed in between 8:00 am to 9:00 am. Similarly, maximum anthesis and pollen dehiscence in morning hours by observed by Pant et al (2003) in *Grewia optiva*, Chowdhuri et al (2004) in *Morus*, Wani (2005) in *Bauhinia variegata* and Chauhan et al (2009) in *Dalbergia sissoo*. Pollen quality is clearly a critical factor in pollination success and the method employed in collecting, storing and testing. The pollen viability percentages of freshly collected pollen were significantly affected by the climatic conditions. In 2021, the maximum viability (93%) was in S7 ecotype followed by S2, S10 and S3, while minimum (78%) pollen

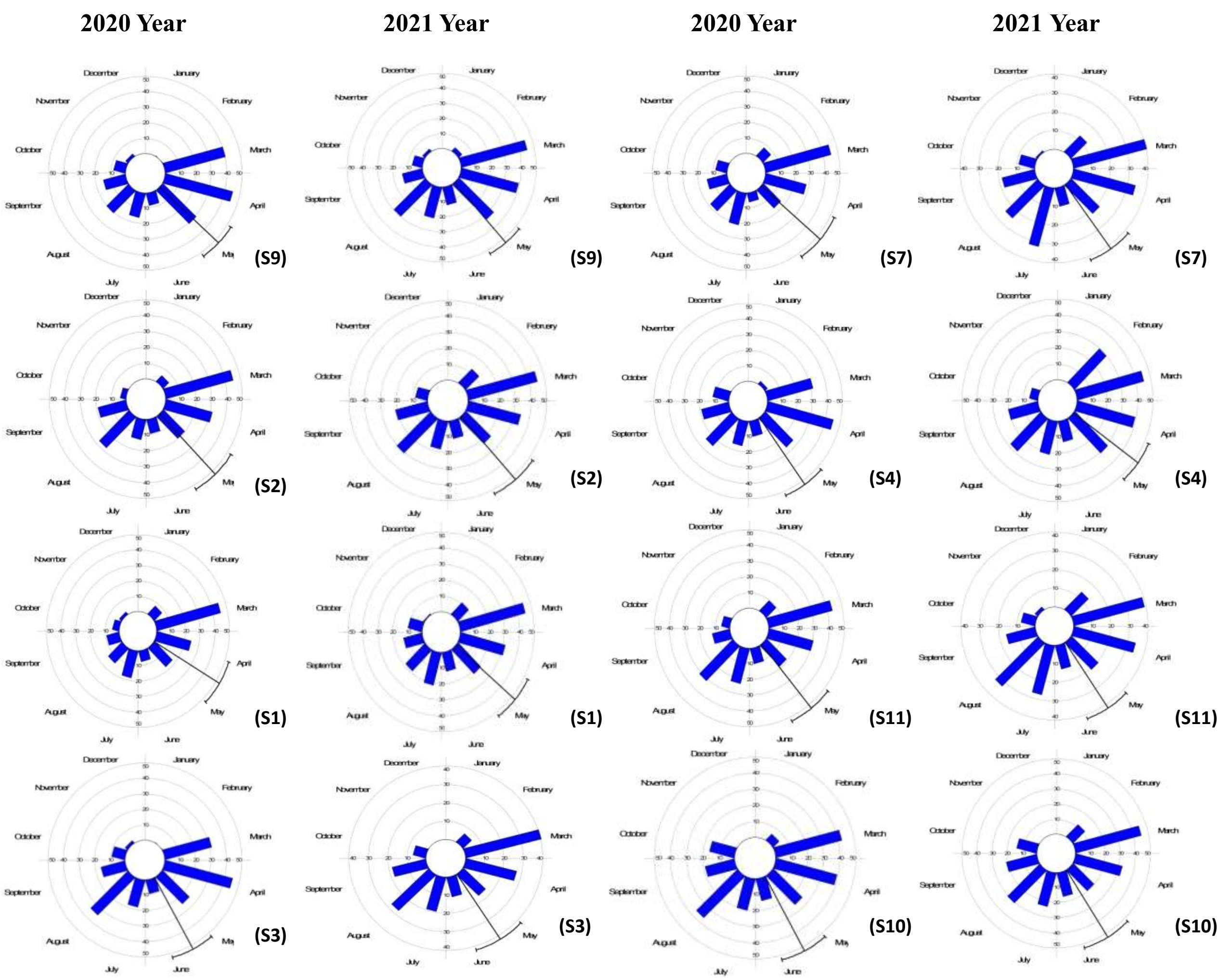

Fig. 3. Circular histograms depicting frequency of vegetative bud swelling dates of *Moringa oleifera* in subtropical climate of north-west India during 2020 and 2021. Bars represent monthly frequency of occurrence an event (vegetative bud swelling) in 45 tagged branches of *M. oleifera* for each year. The line indicates mean seasonal intensity of an event with standard errors

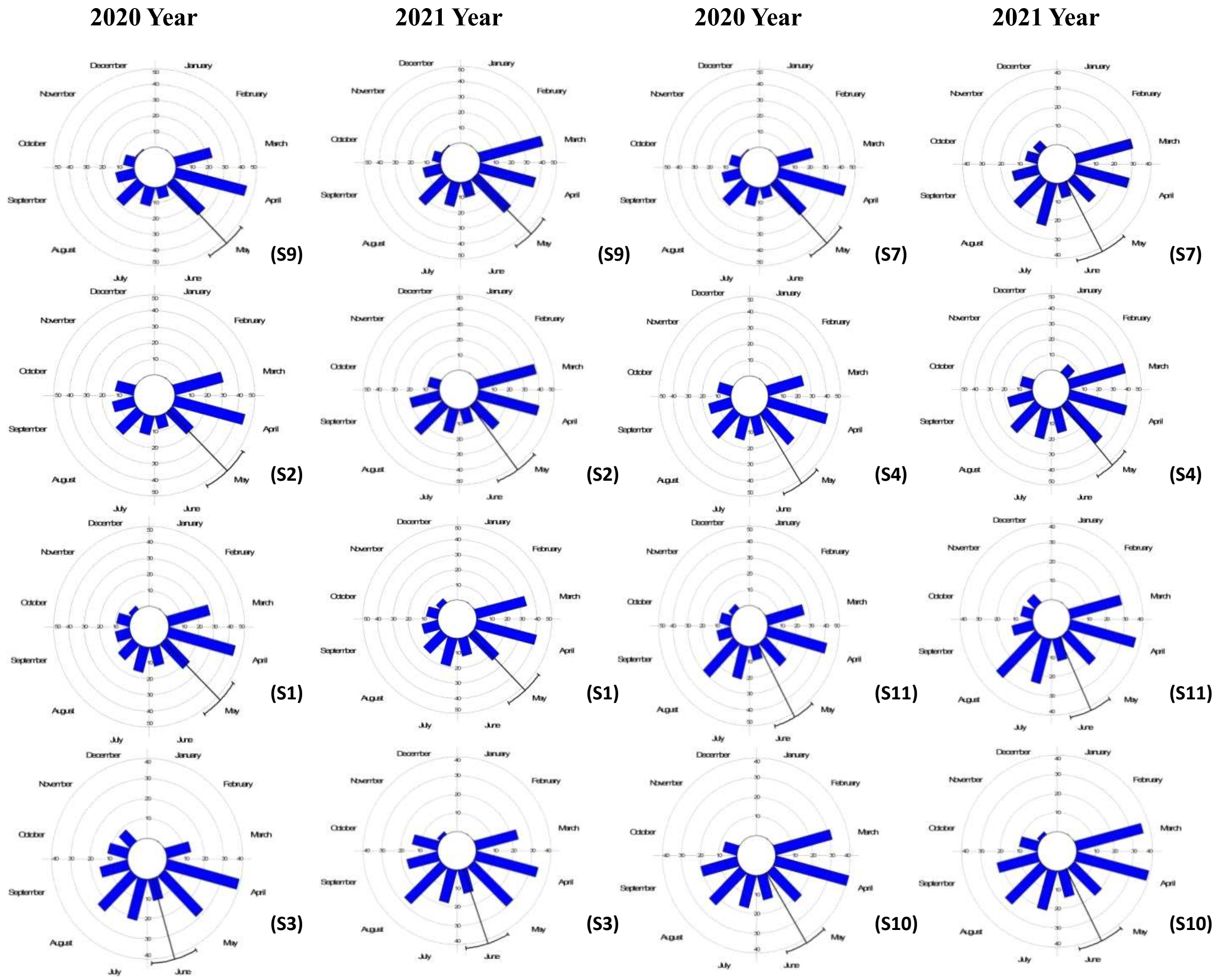

Fig. 4. Circular histograms depicting frequency of vegetative bud bursting dates of *Moringa oleifera* in subtropical climate of north-west India during 2020 and 2021. Bars represent monthly frequency of occurrence an event (vegetative bud bursting) in 45 tagged branches of *M. oleifera* for each year. The line indicates mean seasonal intensity of an event with standard errors

Table 5. Variation in timing of anther dehiscence and pollen studies among different *Moringa oleifera* ecotypes

Moringa ecotypes	Anthers dehiscence	Pollen viability (%) ± SEm	Pollen size (µm)	Pollen germination (%)			
				T2	T3	T4	T5
S9	6.00 hrs-12.30 hrs	80±1.15	45.30×40.23	28±1.15	78±2.31	69±1.15	64±1.15
S7	6.00 hrs-12.00 hrs	93±1.15	46.32×41.05	35±1.15	90±1.15	81±0.58	77±1.15
S2	7.30 hrs-12.30 hrs	88±1.15	43.50×39.45	35±1.15	84±2.31	77±1.15	70±1.15
S4	7.00 hrs-12.00 hrs	78±1.15	47.23×41.12	25±0.58	75±1.73	70±1.15	69±0.58
S1	6.30 hrs-12.00 hrs	80±1.73	45.32×40.18	30±0.58	76±1.73	70±0.58	64±2.31
S11	6.30 hrs-13.00 hrs	82±1.75	46.24×40.87	28±1.51	81±1.24	76±1.41	70±1.42
S3	6.30 hrs-13.00 hrs	83±1.73	46.12×40.65	29±1.73	80±1.15	75±1.73	72±1.15
S10	7.00 hrs-12.30 hrs	85±1.15	44.89×39.86	32±1.15	82±1.15	74±1.15	68±1.15
Mean±Sem	-	83.63±1.35	45.62×40.43	30.25±1.05	80.75±1.85	74.00±1.20	69.25±1.32

Concentration of solution used for pollen germination was 0% (T1), 5% (T2), 10% (T3), 15% (T4) and 20% (T5) sucrose + 200 microgram/ml boric acid.
NO pollen germination was observed in treatment T1 (without sucrose). , Pollen shape, i.e. prolate spheroidal to sub prolate is same in all ecotypes

was observed for S4 local ecotype. Similarly, high percentages (>70%) were earlier recorded by Bhattacharya and Mandal (2004) in *M. oleifera*. The maximum pollen size (47.23×41.12 µm) was in S4 local source followed by pollen S7, S3, S1 and S9 source. Minimum pollen size 43.50×39.45 µm was recorded for S2 source. The pollen size was found to less in subtropical conditions than the *M. oleifera* grown in tropical climate (Bhattacharya and Mandal 2004). Pollen shape was found unaffected by the environmental factors and genotype. All ecotypes had similar type of pollen shape, *i.e.* prolate spheroidal to sub prolate. However, Jyothi et al (1990) reported spheroidal shape pollen grains with (~35 µm) pollen size.

It was excepted that any differences in pollen traits between ecotypes would be seen as in smaller values than local adaptive ecotype of subtropical habitat because of variable weather parameters. However, pollen viability and *in vitro* pollen germination in regional ecotypes were higher in the subtropical habitat. On the other side, pollen size was less than the local cultivar. These pollen traits may be effective for the successful pollination and fertilization under varying level of environment. The mean maximum pollen germination (80.75%) was observed @ 10% sucrose +200 microgram/ml boric acid followed by 15% 20% and 5% sucrose solutions and no germination was recorded for distilled water alone. Pollen germination percentage varied

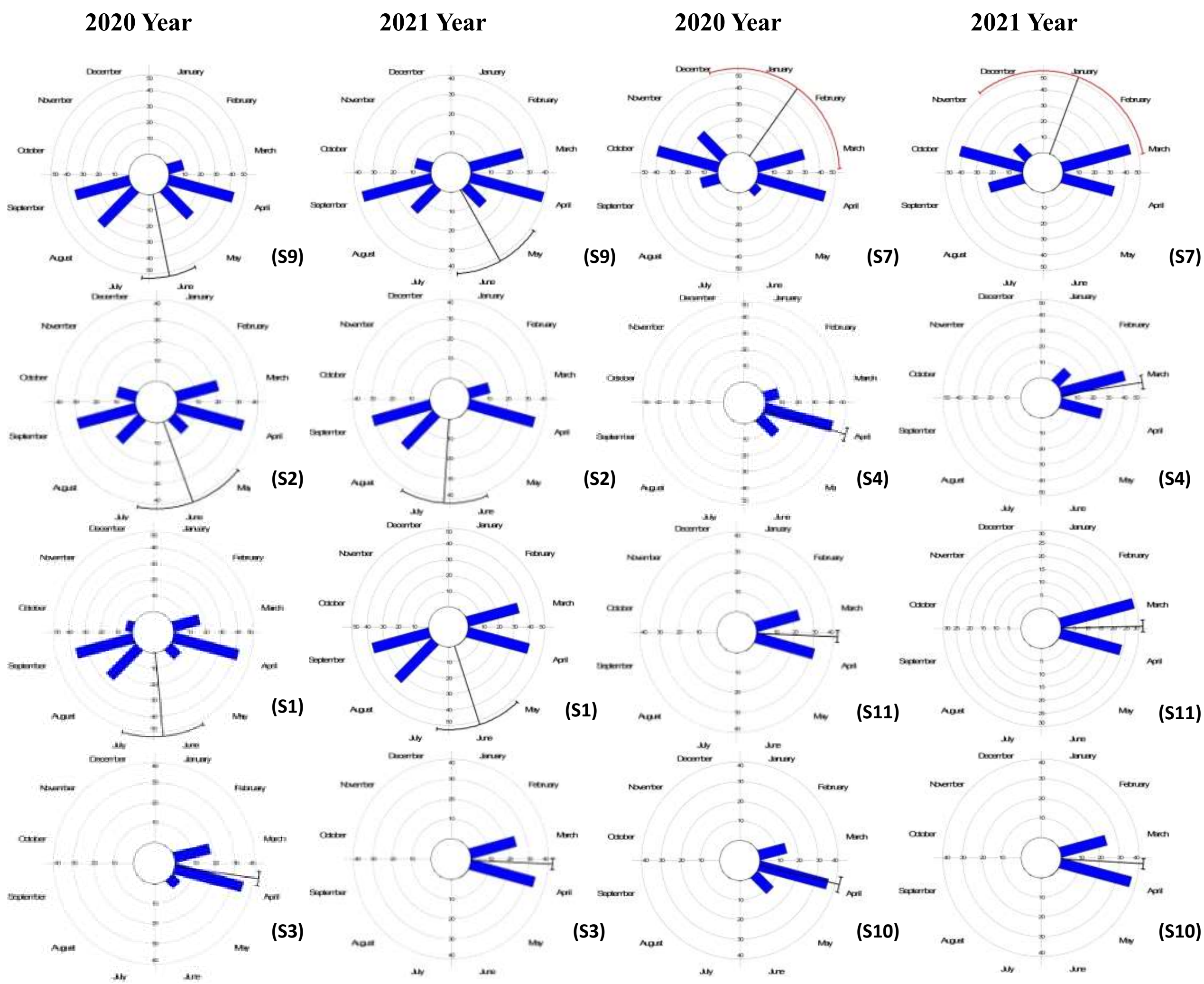

Fig. 5. Circular histograms depicting frequency of floral bud swelling dates of *Moringa oleifera* in subtropical climate of north-west India during 2020 and 2021. Bars represent monthly frequency of occurrence an event (floral bud swelling) in 45 tagged branches of *M. oleifera* for each year. The line indicates mean seasonal intensity of an event with standard errors

between 75-90% @ 10% sucrose + 200 microgram/ml boric acid solution which was higher than S4 local ecotype (Table 5). Pollen grains of S7 source recorded maximum germination (90%) in solution of 10% sucrose supplemented with 200 microgram/ml boric acid followed by S2 (84%), whereas minimum pollen germination percentage (75%) was observed for S4. The present investigations are in agreement with results of Bhattacharya and Mandal (2004) on *M. oleifera* and they reported 94% in vitro pollen germination. Receptivity of stigma is a critical factor governing success of intra- and inter-specific hybridization in dioecious species like *Moringa oleifera*. In order to study the stigma receptivity by visual observation, stigma of different age groups *viz*., 24, 48 and 72 hours after anthesis were observed. The, stigma was considered receptive when the lobes of stigma became fully shining and watery white in colour. The stigmas became receptive between 48 to 72 hours after anthesis. Stigma receptivity among ecotypes varied from three to five days under subtropical climate. Similar observation were reported by Bhattacharya and Mandal (2004) in *M. oleifera*, Pant et al (2003) on *Grewia optiva*, Wani (2005) on *B. variegata* and Chauhan et al (2009) in *D. sissoo*.

Pollination: *Moringa oleifera* is generally insect pollinated plant but combination of insect and wind pollination (Ambophily) had been observed in all *M. oleifera* ecotypes. These insects visited the flowers all the day but maximum activity was observed between 7.00 am to 10.00 am which coincided with the pollen shedding and anthesis period. No

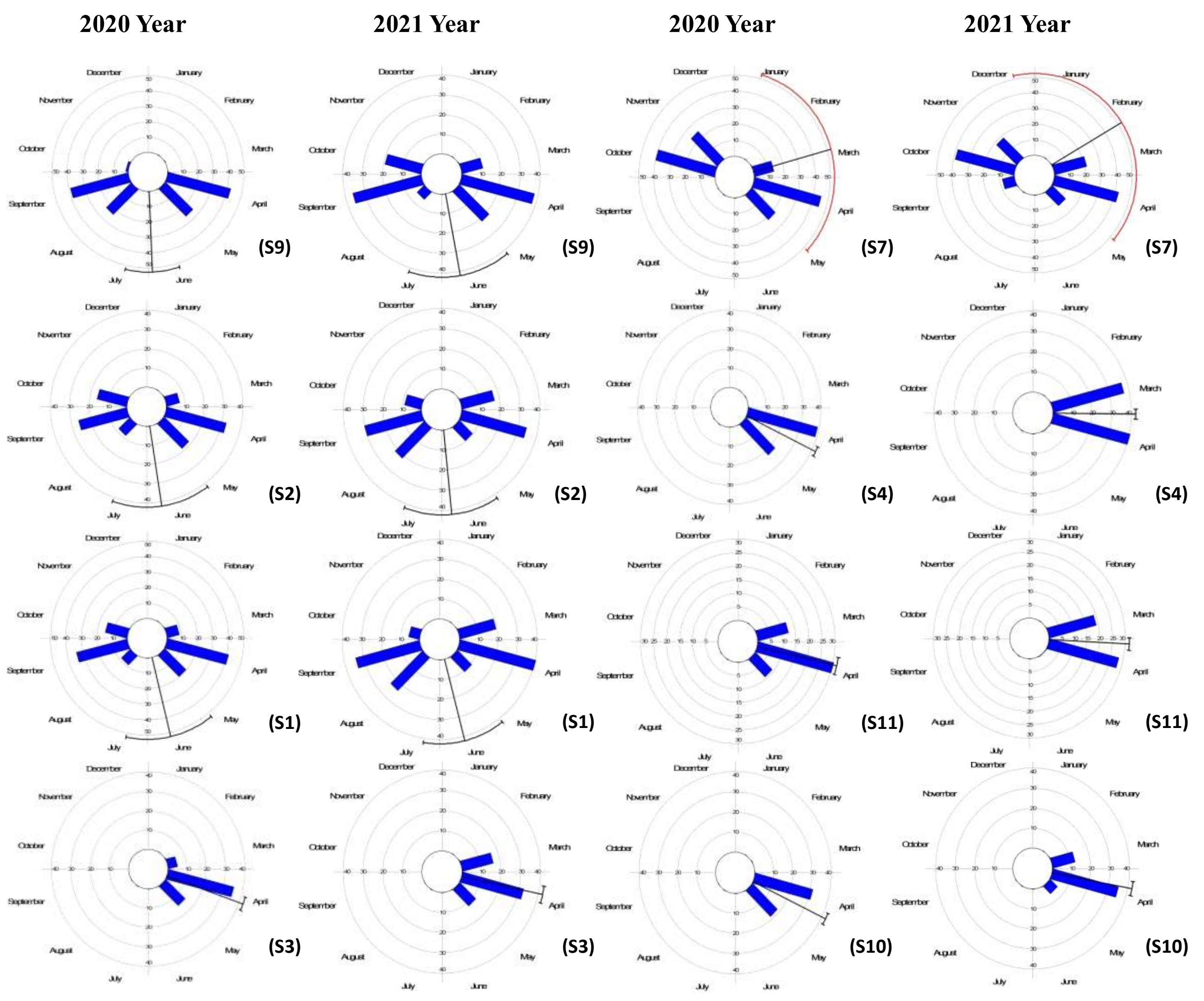

Fig. 6. Circular histograms depicting frequency of floral bud bursting dates of *Moringa oleifera* in subtropical climate of north-west India during 2020 and 2021. Bars represent monthly frequency of occurrence an event (floral bud bursting) in 45 tagged branches of *M. oleifera* for each year. The line indicates mean seasonal intensity of an event with standard errors

clear difference was observed for the selection or preference of particular ecotype. It might be due to similar chemical composition of *Moringa* flowers which is tightly linked with the genetics of plant. The close examination of the mode of pollination revealed that pollen grain sticks to different parts of their body *viz*., abdomen, mouth parts, legs, etc. The visitors affecting cross pollination in *M. oleifera* were western honey bees (*Apies mellifera*), carpenter bee (*Xylocopa* spp.), stingless bee (*Meliponini* spp.), common fruit flies (*Drosophila melanogaster*), wasps (*Polister* spp.) and bumble bee (*Bambus* spp.). Besides, blister beetle (*Mylabris phalerata*), aphids (*Aphis gossypii*), black tree hopper (*Enchenopa binotata*), carpenter ants (*Camponotus* spp.), lady bird beetle (*Coccinellidae* spp.), sun bird (*Leptocoma* Aspasia), Indian myna (*Acridotheres tristis*), Indian palm squirrel (*Funambulus palmarum*) and rose-ringed parakeet (*Psittacula krameri*), of which, flies, wasps, honey bees and bumble bees were the main pollen pollinators. In *Moringa* ecotypes, flowering continues for one and half month and simultaneously development of sweet tinny pods has started. Due to high nutritious pods, large birds and small animal were also spotted on *Moringa* plants. When they climb or jump between branches may carry forward the pollen grains with their body parts and act as a pollinator (Singh et al 2021).

CONCLUSIONS

This is the first report of testing the climate-induced phenological response and reproductive performance of *Moringa oleifera* cultivars and landraces native to tropical, arid and semi-arid agro-ecological regions and grown under subtropical climate of Punjab. Their functional type, growth habit, time of initiation of phenological events, flower morphometrics and pollen characteristics revealed distinct variation induced by seasonal changes in temperature and rainfall pattern as they realized in their native habitat. Vegetative and floral bud characteristics advanced by at least 3 days to 17 days in 2021 than 2020, attributed due to the fluctuation in temperature in spring season and precipitation in winter season. Wider flower dimensions and quality pollen traits in var *PKM-1* (tropical region) and var *Bhagya* (semi-arid region) showed its potentiality for yielding nutritive flowers and pods twice in a year that will likely raise its demand for culinary purposes. Thus, phenological rigidity (earliness of flowering and fruiting) in the evaluated ecotypes will likely strengthen the future breeding strategies in *Moringa oleifera* and had future prospects for promoting climate resilient food production system.

AUTHORS CONTRIBUTION

Conceptualization and designing of the research work (AKD; GPSD); Execution of field/lab experiments (MS, AKD, SS); Data collection (MS); Analysis of data and interpretation (GSW, AKD, RK); Writing - original draft (AKD, MS); Writing - review & editing (AKD, SS). All authors read and approved the paper.

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