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Cold tolerance during early reproductive growth of chickpea (*Cicer arietinum L.*): genetic variation in gamete development and function

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Abstract

Chilling temperatures at flowering cause floral abortion in most chickpea cultivars. Recent evaluations of germplasm showed distinct genotypic differences in pod and seed set at low temperature but the morpho-physiological basis for such variation is unclear. Observations in the field during cold spells of December and January, and at 15/5°C and 15/0°C (day/night) regimes in growth rooms showed distinct genetic variation in flower morphology, gamete development (viability and size of pollen and ovules) and function (pollen germination and tube growth, ovule viability and fertilization, etc.). The greater pod-setting ability of tolerant lines (ICCVs 88502 and 88503) than the sensitive cultivars (Annigeri, Pant G 114, etc.) was associated with a higher pollen vigour (germination and tube growth) and ovule viability at low temperature in the former. The number of ovules was not affected by cold stress in all cultivars/lines but pollen size and viability were reduced in Annigeri. The reduced ovule fertilization, associated with decline in pollen tube growth and ovule viability, was the major cause for poor seed set at low temperatures. The magnitude of effects on gamete function varied with cultivar/line and severity of stress. Function of both pollen and ovules was adversely affected in Annigeri in 15/5° and 15/0°C regimes. In contrast, the reduction in pollen vigour was more than in ovule viability in ICCV 88510 and Pant G 114 at 15/5°C. At 15/0°C, however, both pollen vigour and ovule viability were reduced. A small increase in pod set of sensitive genotypes from manual pollinations with pollen from plants in the warm regime further suggested that pollen function was more adversely affected than pistil function at moderately low temperatures.

Keywords: Chickpea (*Cicer arietinum L.*); Cold tolerance; Gamete development; Pollen; Ovules; Pod set

1. Introduction

Seed production in angiosperms begins with transfer of viable pollen to the stigma and is followed by pollen germination and tube growth, ovule fertilization and seed development. At each step, unfavourable weather conditions impose an upper limit on the number of participating gametes in subsequent processes. The sensitivity of each of these processes may, however, vary with severity of stress and genetic background (Weaver et al., 1984).

The failure of current chickpea cultivars to set pods during cold (day/night temperature regime 15–20/

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<8°C) spells of December and January in sub-tropical northern India (Saxena, 1980; Saxena and Johansen, 1990) is an implicit expression of sensitivity of their reproductive processes to low temperature. Even though a cool-season food legume, chickpea evolved as a spring crop in West Asia, with flowering and podding occurring in progressively increasing temperatures and thus without selection to confer cold tolerance to these processes. Genetic variation in pod set at low temperatures was earlier identified under field conditions (ICRISAT, 1988) and later confirmed both in field and controlled environments (Srinivasan et al., 1998). The morpho-physiological basis for such variation is, however, unclear.

Poor pod set in chickpea can result from the failure of either or both male and female floral parts. Before anthesis, poor pod set may be due to low pollen viability or high ovule sterility. During anthesis to fertilization, it may be due to failure of pollen to reach or germinate on the stigma, or the failure of the pollen tube to penetrate the stigma and grow in the style. Even after fertilization, the developing seed can be aborted either due to a limited supply of photosynthates or disturbance in hormonal balance. Pollen is normally released from chickpea anthers about 15 h before the flower opens (Malti and Shivanna, 1983). Low temperature at flowering can thus delay or reduce anther dehiscence. Savithri et al. (1980) and Jaiwal and Mehta (1983) examined the effects of temperatures above 15°C on in vitro pollen germination and tube growth. The authors observed maximum pollen germination at 25°C and maximum tube growth at 35°C. To our knowledge, the floral and gamete responses of chickpea genotypes to low temperature (0–15°C) have not been determined. The aim of this study was, therefore, to examine the effects of chilling stress both in field and controlled environments, on flower morphology, pollen germination and tube growth, and pistil function of chickpea lines and cultivars with varying cold tolerance in terms of ability to set pods.

2. Materials and methods

In experiment I, 21 chickpea cultivars/lines were evaluated under field conditions during the 1990/1991 season at ICRISAT Cooperative Center, Hisar. Synchronization of flowering with cold spells in December and January was achieved by staggered sowing and/or imposing 18 h long-day treatment. In experiment II, three early (ICCVs 88502, 88503, and Annigeri) and three late (ICCVs 88510, 88514 and Pant G 114) maturing cultivars/lines were then evaluated in the control (25/15°C) and low temperature (15/5° and 15/0°C day/night) regimes in growth rooms. The cultivars/lines used and cultural practices followed in both field and controlled environments, and the light (quality, intensity and photoperiod), temperature, relative humidity and air flow rates at the canopy level are described in detail by Srinivasan et al. (1998). Only the tests to examine gamete development and function are described here.

Under field conditions (Experiment I), flowers were sampled on days when minimum temperature during each of the preceding three nights fell below 5°C. Flowers were chosen from the first 4–5 reproductive nodes on the main branch to permit comparison between flowers of similar physiological growth stage. In each cultivar/line, 20–25 flowers were examined for anther dehiscence on the day of opening. The number of flowers with fully dehiscent anthers and with pollen on stigma was counted, and pollen load on stigma was scored on a 1–5 scale (1, low and 5, high). In growth rooms (Experiment II), at least 10 flowers in each cultivar/line were sampled to measure the size of vexillum, style and ovary, and examine anther dehiscence. The time taken from macroscopic bud appearance to flower opening was also determined. Genotypic differences in gamete development and function were tested at room temperature (22–25°C). Pollen viability was tested on 150–200 pollen grains with 0.5% acetocarmine or Alexander's triple stain (ATS) solution (Alexander, 1969). Germination and tube growth were assessed after incubating pollen in a medium (pH 6.5) containing 10% sucrose, 100 ppm boric acid, 300 ppm calcium nitrate, 200 ppm magnesium sulphate and 100 ppm potassium nitrate (Brewbaker and Kwack, 1963). In addition, genetic variation in germination and tube growth of pollen from plants in the 25/15°C regime was tested after incubating at 0, 5, 10, 15, 20 and 25°C in the dark. The in vitro germination process was stopped at intervals by adding a drop of acetocarmine to the medium. Pollen was counted as germinated when pollen tubes were at least equal to the diameter of

the pollen grain. Percentage germination was determined on the basis of at least 200 grains per replicate. Mean pollen tube length was computed as the average of 20 randomly chosen tubes per replicate.

For in vivo studies, stigmas of flowers were stained on the day of opening with ATS and the numbers of germinated and non-germinated pollen grains were counted. Genetic differences in pollen tube growth were tested after fixing 1-day-old flowers in acetic alcohol (ethanol : acetic acid, 3 : 1, v/v). The gynoecium was removed from the rest of the flower, and transferred to 8 N NaOH for 12 h to soften the tissue. After separating the gynoecium, all operations were carried out in the dark. The styles were placed in cavity blocks filled with 0.1% aniline blue (50 mg aniline blue in 50 ml of 0.1 M trisodium phosphate) for 8–12 h and later mounted in a drop of glycerol. By applying slight pressure on the coverslip, the tissue was spread into a very thin layer and was observed under a Zeiss fluorescence microscope using a barrier filter (515 nm) and an exciter filter BG 12 (320–400 nm). The callose in the pollen tubes fluoresced bright yellow contrasting with the blue of the stylar tissue (Martin, 1959). The number of styles with more than 10 pollen tubes at the base was counted.

An esterase test using α-naphthal acetate as a substrate in the coupling reaction with fast blue B, as modified by Mattson et al. (1974) and Turano et al. (1983), was used for detecting differences in stigma receptivity. Stigmas were removed 1 day before flower opening, immersed in the working solution, and incubated at 37°C for 15 min. A positive test for esterase was indicated by a deep purple stain. Staining intensity was then scored on a 1–5 scale (1, low and 5, high).

The number and size of ovules were measured both in field and controlled environments but viability was determined only in the latter. Ovule viability was tested indirectly through detection of callose (Dumas and Knox, 1983). Flowers, which were initially fixed in acetic-alcohol and stored later in 70% alcohol, were softened with 8 N NaOH for 6 h, rinsed with water and stained with 0.1% aniline blue overnight. Ovules were removed, squashed and observed for fluorescence. The greater the stain, the less was the viability. Percentage fertilization was estimated indirectly on the basis of the number of swollen ovules 3 days after flower opening, as a close association ($r = 0.91^{**}$) between fertilization and swelling of ovules (and enlargement of ovary) was observed earlier (Srinivasan, A., unpublished results).

The effects of low temperature on stigma receptivity and ovule viability were evaluated indirectly in another experiment (Experiment III), by determining pod and seed set following manual pollinations. Plants of Annigeri, ICCV 88510 and Pant G 114 were grown in controlled environment chambers at 25/15°C regime up to anthesis and randomly allocated to 25/15°C, 15/5°C and 15/0°C regimes. The first five opened flowers were removed in all plants to ensure that the flowers to be treated would develop in the respective treatment regimes. Unpollinated flowers were removed to reduce competition in the later stages. In all regimes, a few flowers of each cultivar were manually (a) self-pollinated to test if natural pollen transfer was impaired, and to test if emasculation and pollination had any influence on pod and seed set, and (b) cross-pollinated with pollen from tolerant lines (ICCVs 88502 and 88503) grown in the same thermal regime. In addition, flowers in the 25/15°C regime were fertilized with pollen from cool regimes, and vice versa. Half-open flowers were used for pollination as anthers normally dehisced about 15 h before flower opening (Malti and Shivanna, 1983), and pollen in them was more viable than in fully opened flowers (Eshel, 1968). Anthers were only removed during emasculation without much damage to other floral parts, as the removal of filaments besides anthers increased floral abscission.

Data collection and statistical analysis: For observations on floral morphology in each cultivar/line, at least 20 flowers in field conditions and 10 flowers in growth room conditions were used. In case of observations such as pollen germination and tube growth, data from at least five microscopic fields for each flower were analyzed. In all cases, data were averaged and standard errors of means (SEMs) computed to show the degree of variation within a genotype. The Kruskal–Wallis test was used for comparing different cultivar/line (Steel and Torrie, 1980).

3. Results

3.1. Field experiment (experiment I)

Genotypic variation in reproductive responses to cold stress was substantial under field conditions

Table 1
Genetic variation in anther dehiscence, pollen viability and pollen germination of chickpea during a cold (day/night: 15–20/<8°C) spell in the field during the 1990/1991 season at Hisar

Line/cultivar	Flowers with fully dehiscent anthers (%)	Flowers with pollen on stigma (%)	Pollen load on stigma score[a]	Pollen viability in vitro (%)	Pollen germination	
					In vitro[b]	In vivo[c]
ICCV 88501	85	67	3.2 ± 0.21[d]	91 ± 1.6[d]	79 ± 1.9[d]	39 ± 1.1[d]
ICCV 88502	79	65	3.7 ± 0.13	86 ± 1.9	81 ± 2.1	35 ± 1.7
ICCV 88503	84	77	3.6 ± 0.16	91 ± 1.7	85 ± 2.2	46 ± 1.9
ICCV 88504	72	51	3.1 ± 0.21	79 ± 2.2	63 ± 3.1	12 ± 0.6
ICCV 88505	73	56	2.4 ± 0.19	80 ± 2.4	71 ± 2.7	12 ± 0.8
ICCV 88506	81	79	3.9 ± 0.18	88 ± 1.9	74 ± 2.2	34 ± 2.2
ICCV 88508	73	64	2.1 ± 0.22	79 ± 2.2	53 ± 2.8	11 ± 1.1
ICCV 88509	78	71	3.6 ± 0.24	89 ± 1.7	75 ± 1.7	34 ± 2.0
ICCV 88510	65	61	3.3 ± 0.17	79 ± 2.1	58 ± 2.7	10 ± 0.7
ICCV 88511	74	65	2.6 ± 0.19	91 ± 1.1	63 ± 3.1	35 ± 1.4
ICCV 88512	77	56	3.5 ± 0.22	78 ± 2.4	78 ± 2.9	11 ± 0.9
ICCV 88513	71	61	2.1 ± 0.17	76 ± 2.7	64 ± 2.7	12 ± 0.8
ICCV 88514	72	58	3.1 ± 0.11	79 ± 2.1	66 ± 3.0	16 ± 1.1
ICCV 88515	70	60	2.6 ± 0.21	75 ± 2.4	58 ± 2.1	9 ± 0.7
Pant G 114	62	49	2.7 ± 0.16	79 ± 2.7	58 ± 1.7	10 ± 0.7
Pant G 115	72	63	2.9 ± 0.11	85 ± 2.2	76 ± 2.4	17 ± 1.1
Gaurav	71	58	3.0 ± 0.17	85 ± 1.7	72 ± 2.3	16 ± 1.3
G 130	55	63	2.8 ± 0.19	80 ± 2.7	50 ± 1.9	7 ± 0.5
K 1189	74	75	3.4 ± 0.21	84 ± 2.3	83 ± 2.2	39 ± 2.4
Chafa	63	58	3.0 ± 0.19	56 ± 2.1	39 ± 1.9	6 ± 0.4
Annigeri	59	43	2.8 ± 0.16	60 ± 2.9	51 ± 2.4	6 ± 0.7

[a]Pollen load on stigma on the day of flower opening was scored on a 1–5 scale (1, low and 5, high).
[b]Pollen germination after 2 h culture in Brewbaker and Kwack's medium at room temperature.
[c]Pollen germination on stigma was measured on the day of flower opening.
[d]SEM, cultivar/lines means were significantly different according to the Kruskal–Wallis test.

(Tables 1 and 2). Anther dehiscence, as revealed by the proportion of flowers with fully dehiscing anthers, was maximum in ICCV 88501 and minimum in G 130. More than 75% flowers in ICCVs 88501, 88502, 88503, 88506 and 88509 showed normal anther dehiscence on cool (day/night temperature 15–20/<8°C) days. Among parent lines, only K 1189, a kabuli-type from the former Soviet Union, showed high anther dehiscence. Stigmas in lines such as ICCVs 88502, 88503 and 88506 were well coated with pollen on the day of flower opening. In contrast, pollen load on stigma was much less in cultivars such as Annigeri (Table 1).

Pollen in ICCVs 88501, 88503 and 88511 was more viable (>90%) than in cultivars such as Chafa and Annigeri (≤60%) (Table 1). In vitro pollen germination was lowest in Chafa (39%) and highest in ICCV 88503 (85%). Pollen from K 1189 also germinated well (83%). Pollen germination on the stigma was high in ICCVs 88503, 88501, K 1189, ICCVs 88502 and 88511, in that order, and low in Chafa, Annigeri, G 130, Pant G 114 and ICCV 88515 (≤10%). Ranking of cultivars/lines in terms of pollen germination under in vitro and in vivo conditions was not always similar. For instance, in ICCVs 88505 and 88512, germination was high (71–78%) under in vitro conditions but it was very low (11–12%) on the stigma. It seems that the micro-environment of the stigma influences germination compared to in vitro conditions.

Pollen tube growth in vitro was high in ICCVs 88501, 88502, 88503, 88506 and K 1189 and very low in G 130, Chafa, Annigeri, and ICCVs 88504, 88508 and 88510 (Table 2). The proportion of flowers with more than 10 pollen tubes at the base of the style varied between 62% and 84% in the former group as against only 20–40% in the latter. Esterase activity on the stigma was also high in ICCVs 88501, 88503, 88506 and 88509 (3.8–4.0) and low in Chafa and

Table 2
Genetic variation in pollen tube growth, esterase activity and ovule development and fertilization during a cold (day/night temperature 15–20/<8°C) spell in the field during 1990/1991 season at Hisar

Line/cultivar	Pollen tube growth		Esterase activity score[c]	Ovule development indices (%)[d]		Fertilization of ovules (%)
	In vitro[a] (μm)	In vivo[b] (%)		Number	Size	
ICCV 88501	255 ± 8.9[e]	62(16/26)	4.0 ± 0.14[e]	101	88	59 ± 2.2[e]
ICCV 88502	236 ± 8.3	78(18/23)	3.2 ± 0.22	100	85	56 ± 2.7
ICCV 88503	273 ± 9.2	84(21/25)	3.8 ± 0.17	102	91	64 ± 1.9
ICCV 88504	125 ± 4.1	46(11/24)	3.1 ± 0.23	100	75	37 ± 1.4
ICCV 88505	181 ± 5.9	54(13/24)	3.4 ± 0.19	102	71	33 ± 1.2
ICCV 88506	236 ± 7.7	74(17/23)	3.8 ± 0.17	102	94	49 ± 2.1
ICCV 88508	127 ± 4.4	69(18/26)	3.2 ± 0.22	104	72	35 ± 1.7
ICCV 88509	209 ± 8.5	75(18/24)	3.9 ± 0.21	99	90	49 ± 2.3
ICCV 88510	128 ± 4.7	36(9/25)	3.0 ± 0.19	102	75	43 ± 2.4
ICCV 88511	144 ± 5.3	46(11/24)	3.4 ± 0.17	103	91	33 ± 1.7
ICCV 88512	165 ± 6.3	58(15/26)	3.1 ± 0.22	98	85	40 ± 2.0
ICCV 88513	202 ± 9.7	63(15/24)	3.2 ± 0.18	102	83	31 ± 1.7
ICCV 88514	182 ± 8.2	42(10/24)	2.9 ± 0.23	105	77	35 ± 1.3
ICCV 88515	148 ± 6.3	30(7/23)	3.1 ± 0.19	98	80	35 ± 1.7
Pant G 114	148 ± 8.1	38(10/26)	2.8 ± 0.23	101	69	23 ± 1.1
Pant G 115	206 ± 8.7	37(10/27)	3.4 ± 0.21	103	79	30 ± 1.4
Gaurav	201 ± 8.6	48(13/27)	3.1 ± 0.22	99	81	28 ± 1.1
G130	119 ± 5.2	37(10/27)	2.8 ± 0.18	101	72	24 ± 1.8
K 1189	245 ± 9.6	76(19/25)	3.2 ± 0.19	102	79	45 ± 2.1
Chafa	120 ± 5.4	22(6/27)	1.6 ± 0.15	96	64	19 ± 1.4
Annigeri	123 ± 5.1	24(6/25)	1.8 ± 0.21	97	56	18 ± 1.1

[a]Pollen tube growth was measured after 2 h culture in Brewbaker and Kwack's medium at room temperature.
[b]Percentage of flowers with at least 10 pollen tubes at the base of styles 1 day after flower opening. Figures in parentheses: (flowers with >10 pollen tubes at the base of the style/total flowers tested).
[c]Esterase activity on stigma was scored on a 1–5 scale (1, low and 5, high) 1 day before flower opening.
[d]Ovule development (number and size) in flowers which opened on days when temperatures during each of the preceding three nights fell below 5°C for at least 6 h expressed as % of that in flowers which opened on days when minimum temperatures were above 12°C.
[e]SEM, cultivar/lines means were significantly different according to the Kruskal–Wallis test.

Annigeri (1.6–1.8). However, even cold-sensitive cultivars such as Pant G 115 and Gaurav showed moderately high levels of esterase activity (3.1–3.4).

The number of ovules per ovary was largely unaffected by cold stress in all cultivars/lines but the size of ovules in flowers opened on cool (day/night temperature 15–20/<8°C) days was 9–45% less than that in flowers opened on warm (day/night temperature 22–25/>10°C) days (Table 2). The size of ovules was reduced most in lines/cultivars such as Annigeri, Chafa, Pant G 114, ICCVs 88505 and 88508. In contrast, the decrease in ovule size in ICCVs 88501, 88503 and 88506 was marginal. The size of ovules in ICCV 88511, which had relatively low levels of pollen load on stigma and in vitro pollen germination and tube growth, was largely unaffected by cold stress. About 55–60% ovules were fertilized in the tolerant (ICCVs 88501, 88502 and 88503) lines as against 18–25% in the sensitive (Annigeri, Chafa, Pant G 114, G 130, etc.) group.

3.2. Experiments in growth rooms (Experiment II and III)

3.2.1. Genetic variation in germination and tube growth responses of pollen to incubation temperature

In all cultivar/lines, incubation temperature greatly influenced both germination and tube growth of pollen from plants in the control regime (Table 3). Essentially, pollen in all cultivars/lines failed to germinate and grow at 0°C. Genetic variation was, however, distinct from 5°C to 20°C. Pollen from ICCVs 88502 and 88503 germinated more than pollen from

Table 3
Genetic variation in pollen germination and tube growth in vitro (±SE) after 3 h of culture at various incubation temperatures

Temperature (°C)	Cultivar/line					
	Annigeri	ICCV 88502	ICCV 88503	Pant G 114	ICCV 88510	ICCV 88514
			Pollen germination (%)			
0	0.3 ± 0.1	1.8 ± 0.1	1.1 ± 0.1	1.4 ± 0.2	1.2 ± 0.1	1.1 ± 0.1
5	4 ± 0.1	11 ± 0.4	14 ± 0.8	6 ± 0.3	8 ± 0.4	7 ± 0.3
10	10 ± 0.7	34 ± 2.2	38 ± 1.8	17 ± 1.1	19 ± 1.3	19 ± 1.4
15	22 ± 1.6	47 ± 1.9	50 ± 1.7	31 ± 1.4	34 ± 1.6	36 ± 1.8
20	49 ± 1.9	64 ± 2.2	68 ± 2.4	55 ± 2.1	59 ± 1.7	62 ± 2.5
25	81 ± 2.2	77 ± 1.9	83 ± 1.6	78 ± 2.7	81 ± 2.9	84 ± 2.7
			Pollen tube growth (μm)			
0	3.5 ± 0.14	11 ± 0.9	12 ± 0.9	11 ± 0.8	14 ± 1.1	14 ± 1.2
5	28 ± 2.4	76 ± 3.4	92 ± 3.2	65 ± 1.7	62 ± 2.4	71 ± 1.9
10	81 ± 3.7	191 ± 5.3	225 ± 6.1	142 ± 4.2	161 ± 6.7	173 ± 5.3
15	239 ± 11.5	495 ± 9.2	563 ± 11.4	414 ± 7.8	441 ± 9.4	497 ± 10.2
20	714 ± 14.7	1094 ± 11.9	919 ± 10.3	688 ± 10.9	747 ± 11.7	912 ± 13.2
25	1086 ± 18.1	986 ± 14.7	1015 ± 15.8	994 ± 13.2	1064 ± 15.7	952 ± 14.3

others at 5°C, 10°C and 15°C. For instance, only 22% pollen germinated in Annigeri as against 50% in ICCV 88503 at 15°C. However, the differences in pollen germination among the late maturing cultivars/lines were not distinct. A similar trend was noticed in pollen tube growth but pollen tubes of ICCVs 88510 and 88514 grew much longer than those of Pant G 114 at 10°C, 15°C and 20°C. A few structural differences in pollen tube growth were also found. Tube growth at low temperatures was more uniform in ICCVs 88502 and 88503 than in others. In Annigeri, Pant G 114 and ICCV 88510, pollen tubes were distorted around tips especially at 5°C and 10°C (data not shown).

3.2.2. *Genetic variation in floral morphology, gamete development and function in low temperature regimes*

The duration from macroscopic bud appearance to flower opening was longer in cool regimes than in the control regime for all cultivars/lines (Table 4). The delay was more in Annigeri (2.6 days) than in others (0.6–1.2 days) in the 15/0°C regime. The variation in late maturing cultivars/lines was, however, marginal in all the regimes. Vexillum size was largely unaffected by low temperature in all cultivars/lines, albeit a small but non-significant decrease in width was seen in Annigeri and ICCV 88502 (Table 4). The size of style were reduced in Annigeri, Pant G 114 and ICCV 88510, and ovary size in Annigeri especially at the 15/0°C regime (Table 4). Because of this, the distance between the levels of anther and stigma on the day of flower opening was more in these cultivars than in others (data not shown).

All flowers in the control regime had fully dehiscing anthers at the time of opening. In the 15/5°C regime, the proportion of flowers with fully dehiscing anthers was 71% in Annigeri as against 87–94% in others. Anther dehiscence was very low in the 15/0°C regime in all cultivars/lines, but much less in Annigeri (35%) and Pant G 114 (69%) than in others (75–81%). The number of partially dehiscent or indehiscent anthers was especially high in Annigeri in both 15/5 and 15/0°C regimes. In the control regime, copious amounts of pollen were released after tapping anthers and stigmas were covered with pollen at the time of flower opening. In contrast, small amounts of pollen were released and the number of flowers with pollen on stigmas was low in Annigeri in the 15/5°C regime. In the 15/0°C regime, however, the proportion of flowers with pollen on stigma was reduced in all cultivars/lines but the magnitude of reduction was less in ICCVs 88502 and 88503 than in others. The adverse effects of cold stress on anther dehiscence in the 15/0°C regime were noticed more in the later-opened flowers than in the earlier ones, especially in Annigeri (data not shown). Esterase activity on the stigma was highest in all cultivars/lines except Annigeri in the 15/5°C regime. It remained high in ICCVs 88502 and 88503,

Table 4
Effects of cold stress on floral morphology, anther dehiscence and esterase activity (± SE) in chickpea cultivars/lines grown in growth rooms

Thermal regime (day/night °C)	Cultivar/line					
	Annigeri	ICCV 88502	ICCV 88503	Pant G 114	ICCV 88510	ICCV 88514
	Duration from bud appearance to flower opening (days)[a]					
25/15	5.6 ± 0.16	6.1 ± 0.18	5.8 ± 0.15	6.0 ± 0.16	6.1 ± 0.18	6.1 ± 0.21
15/5	7.2 ± 0.21	6.7 ± 0.17	6.4 ± 0.17	6.3 ± 0.13	6.4 ± 0.19	6.7 ± 0.17
15/0	8.2 ± 0.19	6.7 ± 0.21	6.6 ± 0.17	7.2 ± 0.15	7.0 ± 0.18	7.2 ± 0.19
	Width of vexillum (mm)					
25/15	6.5 ± 0.21	6.8 ± 0.21	6.7 ± 0.25	7.1 ± 0.23	6.8 ± 0.21	7.0 ± 0.20
15/5	6.1 ± 0.20	6.5 ± 0.21	6.7 ± 0.21	7.2 ± 0.19	6.7 ± 0.22	7.1 ± 0.17
15/0	6.3 ± 0.24	6.5 ± 0.23	6.8 ± 0.24	7.2 ± 0.21	7.0 ± 0.19	6.7 ± 0.21
	Length of style (mm)					
25/15	6.4 ± 0.18	6.6 ± 0.21	6.4 ± 0.22	6.9 ± 0.19	6.5 ± 0.22	6.7 ± 0.16
15/5	5.7 ± 0.19	6.5 ± 0.17	6.2 ± 0.18	6.1 ± 0.21	6.6 ± 0.11	6.7 ± 0.19
15/0	4.4 ± 0.16	6.3 ± 0.19	6.3 ± 0.19	5.7 ± 0.22	5.9 ± 0.14	6.4 ± 0.18
	Length of ovary (mm)					
25/15	1.9 ± 0.07	2.0 ± 0.08	1.8 ± 0.07	2.0 ± 0.07	1.9 ± 0.08	1.9 ± 0.07
15/5	1.4 ± 0.06	2.1 ± 0.08	1.9 ± 0.06	2.0 ± 0.06	2.0 ± 0.07	1.8 ± 0.07
15/0	1.3 ± 0.06	1.9 ± 0.07	1.8 ± 0.05	1.9 ± 0.07	1.9 ± 0.07	2.0 ± 0.08
	Flowers with fully dehiscing anthers (%)					
25/15	100(17/17)[b]	100(15/15)	100(14/14)	100(15/15)	100(15/15)	100(16/16)
15/5	71(11/17)	93(14/15)	94(16/17)	87(13/15)	88(14/16)	88(15/17)
15/0	35(6/17)	75(12/16)	81(13/16)	69(11/16)	76(13/17)	82(14/17)
	Partially dehiscent or indehiscent anthers per 10 flowers (no.)					
25/15	0	0	0	0	0	0
15/5	26	5	4	7	8	6
15/0	41	9	7	19	15	12
	Flowers with pollen on stigma (%)					
25/15	100(17/17)	100(15/15)	100(14/14)	100(15/15)	100(15/15)	100(16/16)
15/5	65(11/17)	93(14/15)	100(17/17)	100(15/15)	88(14/16)	88(15/17)
15/0	18(3/17)	81(13/16)	88(14/16)	69(11/16)	71(12/17)	65(11/17)
	Esterase activity on stigma[c]					
25/15	4.8 ± 0.07	4.9 ± 0.08	4.8 ± 0.08	4.9 ± 0.07	4.8 ± 0.08	4.9 ± 0.07
15/5	2.3 ± 0.19	4.1 ± 0.18	4.0 ± 0.15	4.2 ± 0.18	3.9 ± 0.15	4.1 ± 0.11
15/0	1.6 ± 0.15	3.8 ± 0.21	3.8 ± 0.17	3.2 ± 0.16	3.4 ± 0.17	3.2 ± 0.16

[a]The buds, which failed to open especially in the 15/0°C regime, were not considered in calculations.
[b]Figures in parentheses: (flowers with only dehiscing anthers or with pollen on stigma/total number of flowers tested).
[c]Esterase activity was scored on a 1–5 scale (1, low and 5, high) 1 day before flower opening.
Note: cultivar/line means were significantly different according to the Kruskal–Wallis test.

moderate in all late-maturing cultivars/lines, and lowest in Annigeri in the 15/0°C regime (Table 4).

Two classes of pollen were differentiated. Prior to staining, non-viable pollen appeared small, round in shape, and aggregated, while viable pollen appeared large, oblong to irregular in shape and dispersed. After staining with acetocarmine, fertile pollen stained uniformly and had a triangular shape, while sterile pollen grains were round in shape and did not take up any stain. The acetocarmine test revealed at least four types of pollen: plump and deeply stained, plump and partially stained, shrunken and partially stained, and shrunken and unstained. The first two groups were considered viable and the latter two, non-viable. With

Table 5
Effects of cold stress on development and function of pollen and ovules (± SE) in chickpea cultivars/lines grown in growth rooms

Thermal regime (day/night °C)	Cultivar/line					
	Annigeri	ICCV88502	ICCV 88503	Pant G 114	ICCV 88510	ICCV 88514
			Pollen grain size (μm)			
25/15	27 ± 0.41	29 ± 0.42	29 ± 0.57	30 ± 0.32	29 ± 0.31	31 ± 0.46
15/5	26 ± 0.52	28 ± 0.44	29 ± 0.41	31 ± 0.37	30 ± 0.34	30 ± 0.47
15/0	24 ± 0.71	28 ± 0.53	29 ± 0.47	29 ± 0.39	30 ± 0.43	30 ± 0.52
			Pollen viability (%)			
25/15	91 ± 1.8	94 ± 1.7	92 ± 1.8	94 ± 1.4	93 ± 1.6	94 ± 1.4
15/5	78 ± 2.1	90 ± 2.2	86 ± 2.1	89 ± 1.9	91 ± 1.8	90 ± 1.1
15/0	59 ± 2.8	89 ± 1.9	87 ± 2.3	79 ± 2.4	82 ± 2.2	84 ± 1.8
			Pollen germination in vitro (%)			
25/15	87 ± 1.9	86 ± 2.1	87 ± 1.9	89 ± 1.7	87 ± 1.9	84 ± 1.7
15/5	53 ± 2.2	64 ± 1.8	68 ± 1.7	59 ± 2.2	59 ± 2.2	63 ± 1.6
5/0	32 ± 1.7	58 ± 1.8	61 ± 2.1	47 ± 1.9	52 ± 1.8	53 ± 1.9
			Pollen germination in vivo (%)			
25/15	68 ± 1.8	71 ± 2.1	64 ± 1.9	67 ± 2.2	70 ± 2.2	62 ± 1.8
15/5	19 ± 1.1	51 ± 1.7	55 ± 1.6	23 ± 0.6	27 ± 1.3	22 ± 1.1
15/0	9 ± 0.6	33 ± 0.9	47 ± 1.5	15 ± 0.8	12 ± 0.8	14 ± 0.8
			Pollen tube growth in vitro (%)			
25/15	946 ± 16.3	981 ± 15.1	878 ± 14.2	1064 ± 17.1	951 ± 14.3	1015 ± 13.8
15/5	238 ± 9.2	595 ± 11.3	663 ± 12.1	414 ± 11.3	447 ± 11.7	481 ± 9.6
15/0	81 ± 3.8	221 ± 4.7	299 ± 9.1	172 ± 6.5	184 ± 7.2	167 ± 5.8
			Pollen tube growth in vivo (%)			
25/15	100	100	100	100	100	100
15/5	23.5(4/17)[a]	76.5(13/17)	82.3(14/17)	55.6(10/18)	58.8(10/17)	61.1(11/18)
15/0	17.6(3/17)	52.9(9/17)	55.6(10/18)	31.3(5/16)	35.3(6/17)	38.8(7/18)
			Ovules per ovary (no.)			
25/15	2.5 ± 0.13	2.4 ± 0.11	2.4 ± 0.13	2.7 ± 0.14	2.6 ± 0.13	2.8 ± 0.17
15/5	2.4 ± 0.11	2.3 ± 0.15	2.4 ± 0.17	2.6 ± 0.13	2.4 ± 0.15	2.7 ± 0.12
15/0	2.5 ± 0.17	2.4 ± 0.14	2.4 ± 0.12	2.7 ± 0.17	2.6 ± 0.12	2.8 ± 0.14
			Maximum size of ovule at flower opening (μm)			
25/15	452 ± 5.5	435 ± 5.1	444 ± 4.7	493 ± 5.8	514 ± 5.2	484 ± 4.7
15/5	366 ± 4.1	363 ± 4.4	392 ± 5.3	417 ± 3.8	423 ± 3.9	407 ± 5.2
15/0	334 ± 4.7	377 ± 4.1	402 ± 4.6	425 ± 4.7	413 ± 4.1	412 ± 5.4
			Viability of ovules at flower opening (%)			
25/15	81 ± 1.8	86 ± 1.7	84 ± 2.2	85 ± 1.5	84 ± 1.7	88 ± 1.8
15/5	43 ± 1.7	55 ± 1.8	67 ± 1.6	45 ± 1.9	58 ± 2.2	53 ± 1.7
15/0	17 ± 1.1	49 ± 2.2	59 ± 2.1	28 ± 1.8	31 ± 1.6	32 ± 2.1
			Ovule fertilization (%)			
25/15	85 ± 1.9	82 ± 2.4	84 ± 2.2	87 ± 2.1	88 ± 1.7	83 ± 2.9
15/5	8 ± 0.2	42 ± 1.2	49 ± 1.7	19 ± 0.6	21 ± 1.3	26 ± 1.1
15/0	2 ± 0.1	23 ± 1.1	34 ± 1.1	8 ± 0.2	9 ± 0.3	12 ± 0.4

[a]Figures in parentheses: (styles with >10 pollen tubes at the base/total flowers tested).
Note: cultivar/line means were significantly different according to the Kruskal–Wallis test.

ATS, viable pollen (27–32 μm) stained red and the non-viable (16–24 μm) ones green. Grain size was largely unaffected by low temperature in all cultivars/lines except Annigeri, in which pollen grains were smaller by 12% in the 15/0°C than in the control regime. Pollen viability was reduced in all cultivars in the 15/0°C regime but the magnitude of reduction was higher in Annigeri (41%) and Pant G 114 (21%) than in others (11–16%) (Table 5).

In all cultivars/lines, in vitro germination of pollen from plants in cool regimes was less than in the control regime (Table 5). After staining stigmas with ATS at flower opening, the germinated pollen grains appeared 'empty' and green, as against viable but non-germinated grains, which appeared red. Germination in vivo was slow and sporadic in all cultivars/lines in cool regimes. In the control regime, pollen germinated within 30 min after pollination as against 2–3 h in 15/5°C and 15/0°C regimes. Pollen germination in vivo was much less than under in vitro in all regimes. In the 15/5°C regime, 51–55% pollen germinated on the stigma in ICCVs 88502 and 88503 as against only 19–27% in others. In the 15/0°C regime, however, 47% pollen was germinated in ICCV 88503 as against only 9% at Annigeri (Table 5).

In all cultivars/lines (Table 5), pollen tubes grew faster and more uniformly in the control than in cool regimes. In the 15/5°C regime, pollen tube growth was high in ICCVs 88502 and 88503, moderate in late-maturing genotypes, and very low in Annigeri. A similar trend was noticed in the 15/0°C regime but the effects were more drastic. Pollen in the 15/0°C regime exhibited several abnormalities during germination. A few pollen grains, instead of germinating, burst and their contents exuded. In others, pollen tubes showed various degrees of bends, branching, waviness, bloating, and blunt tips. All flowers sampled in the control regime had more than 10 pollen tubes at the base of the style. In contrast, only 24% of flowers in Annigeri showed such pollen tube growth in the 15/5°C regime. About 53–55% of flowers in ICCVs 88502 and 88503 had similar levels of tube growth in the 15/0°C regime.

The number of ovules was unaffected by cold stress. However, ovule size and viability were reduced in all cultivars/lines in cool regimes. For instance, ovule size decreased by 10% in ICCV 88503 and 14–28% in others in the 15/0°C regime. The reduction in ovule viability, as indicated by an increased callose deposition, was more in other cultivars/lines than in ICCVs 88502 and 88503 (Table 5). Ovule shrivelling or collapse was also seen in the cool regimes, especially in Annigeri in the 15/0°C regime. Even the embryo sac was absent in ovules of the late-opened flowers in Annigeri (data not shown). The proportion of fertilized ovules was reduced in cool regimes in all cultivars/lines but the reduction was more in Annigeri, Pant G 114, and ICCV 88510 (<10% in the 15/0°C regime) than in others (Table 5).

Manual pollinations in the control regime with pollen from plants in the same regime resulted in good pod and seed set in all cultivars/lines (Table 6). Similar pollinations in the 15/5°C regime resulted in no pod set in Annigeri, and 3–6% pod set in ICCV 88510 and Pant G 114. All pods were, however, seedless. In the 15/0°C regime, no pod or seed set was observed in any of the cultivars/lines. When pollen from cool regimes was used to pollinate flowers in the warm regime, a substantial reduction in pod and seed set as compared to that resulting from pollen in the control regime was observed (Table 6). Pod and seed set from these pollinations were, however, more than those from self-pollinations in cool regimes. Pod set was less in Annigeri in both 15/5°C and 15/0°C regimes than in ICCV 88510 and Pant G 114. Further, all pods set in Annigeri following pollinations with pollen from 15/0°C were seedless (Table 6).

When pollen from the control regime was used to pollinate flowers in the 15/5°C regime, pod and seed set were moderate in Pant G 114 and very low in Annigeri (Table 6). Pods in both Annigeri and ICCV 88510 were without seeds. Pollinations from the control regime had no effect on pod and seed set in the 15/0°C regime except Pant G 114, which set a few seedless pods.

Pollen from ICCV 88503 gave a higher pod set than pollen from ICCV 88502 in all regimes (Table 6). When pollen from ICCV 88502 was used to pollinate flowers in the 15/5°C regime, there was an increase in pod set in all cultivars/lines but the increase was more in ICCV 88510 than in Pant G 114 and Annigeri. In the 15/0°C regime, however, no pod and seed set were observed with pollen from ICCV 88502 in all cultivars/lines. Pollination with pollen from ICCV 88503 increased pod set in ICCV 88510 and Pant G114 in the 15/0°C regime but all pods were seedless (Table 6).

Table 6
Effects of pollen source on pod set and pods with at least one seed (seeded pods) in chickpea cultivars/lines grown in various thermal regimes

Female cultivar/line	Pollen source														
	Own pollen from									Tolerant lines in the same regime					
	25/15°C regime			15/5°C regime			15/0°C regime			1ICCV 88502			ICCV 88503		
	Pol. no.[a]	Pod set (%)	Seeded pods (%)	Pol. no.	Pod set (%)	Seeded pods (%)	Pol. no.	Podset (%)	Seeded pods (%)	Pol. no.	Pod set (%)	Seeded pods (%)	Pol. no.	Pod set (%)	Seeded pods (%)
25/15°C regime															
Annigeri	32	72	66	24	15	10	24	7	0	29	72	65	25	76	68
ICCV 88510	24	75	67	30	27	27	26	15	12	31	77	71	30	74	68
Pant G 114	28	79	75	25	34	20	30	13	13	26	77	69	30	77	70
15/5°C regime															
Annigeri	24	10	0	35	0	0	32	0	0	31	3	0	26	4	0
ICCV 88510	26	19	0	34	3	0	35	0	0	34	18	9	32	19	6
Pant G 114	24	29	14	35	6	0	35	0	0	32	16	6	28	21	11
15/0°C regime															
Annigeri	24	0	0	30	0	0	26	0	0	30	0	0	25	0	0
ICCV 88510	28	0	0	32	0	0	24	0	0	34	3	0	28	7	0
Pant G 114	24	6	0	31	0	0	28	0	0	32	0	0	29	7	0

[a]Pol. no. refers to the number of artificial pollinations made.

4. Discussion

The results showed that genetic variation in floral and gamete responses of chickpea to cold (day/night 15–20/<8°C) stress was substantial both in field and controlled environments. In sensitive cultivars/lines, the size of the vexillum was largely unaffected but sizes of ovary and style were reduced and floral development was delayed, especially in the 15/0°C regime, perhaps due to low rates of metabolism. The reduction in pistil size has many implications for pod and seed set. Because of the reduced pistil size, the distance between the levels of anther and stigma at the time of flower opening was greater in sensitive than in tolerant cultivars/lines, thereby reducing the chances of pollen transfer to stigma. Such phenomenon of heterostyly induced by cold stress can limit pollination and fertilization, even if pollen remains viable and germinable.

Anther dehiscence and pollen load on stigma were reduced by cold stress in all cultivars/lines. The extent of reduction was, however, less in tolerant (ICCVs 88502 and 88503) than in sensitive (Annigeri and Pant G 114) cultivars/lines (Tables 1, 2 and 4). Qian et al. (1986) and Xu et al. (1990) reported that duration of pollen maturation and anther dehiscence were indicative of rates of cell division in gametophytic tissue. Genetic variation in anther dehiscence may, therefore, be associated with differences in (a) rates of cell division in gametophytic tissue and/or (b) formation of endothecium at low temperatures. The release of small amounts of pollen after tapping anthers in sensitive cultivars/lines in cool regimes suggested that chilling stress adversely affected pollen release and pollination.

Fitness or transmission ability of pollen depends on both development (growth, viability, and competition within the anther) and function (germination, tube growth and fertilization ability). Pollen development (size and viability) was largely unaffected by cold stress under field conditions in most cultivars/lines (Table 1), thus, indicating a relative insensitivity of meiosis in the male gametophyte to moderately low temperatures. Brooking (1976) reported that pollen viability was not reduced by low temperature in sorghum (*Sorghum bicolor (L.) Moench*) if meiosis had progressed beyond leptotene. Both size and viability of pollen were, however, reduced in cultivars/lines such as Annigeri perhaps because of a higher sensitivity of one or more stages in meiosis to cold stress and/or failure of pollen to mature at low temperature as observed in rice (*Oryza sativa L.*) (Ito et al., 1970). A comparative study of pollen cytology in plants in the control and cool regimes with treatments applied over much shorter times is necessary to determine the mechanisms regulating pollen developmental responses. A greater reduction in pollen viability in the 15/0°C than in the 15/5°C regime, and similar levels of pollen sterility in the field and the 15/0°C regime in Annigeri suggested that the critical minimum night temperature affecting viability in this genotype may be between 0 and 5°C.

Competitive ability in early stages of pollen function is known to be governed by sporophytic tissues, which control the amount of storage material in the pollen grain. This amount supports only the first stage of pollen tube growth (autotrophic development). The later growth stage, which depends on metabolites provided by the style (heterotrophic development), is largely controlled by gametophytic tissues (Heslop-Harrison et al., 1984). Genetic variation in pollen function can thus arise from differences in sensitivity of both sporophytic and gametophytic tissues to cold stress. The reduced germinability of pollen in sensitive cultivars/lines may be due to the smaller amount of storage material in pollen grains than in tolerant ones, which in turn may be associated with direct effects of low temperature on deposition or synthesis of reserves. The loss of membrane integrity at low temperature may be another factor (Heslop-Harrison et al., 1984). Because turgidity is an absolute requirement for germination, a cell with leaking membranes cannot become turgid and germinate. Abnormal digestion of starch in pollen grains at low temperature as observed in rice (Koike and Satake, 1987) may also contribute to the loss of germinability in sensitive cultivars/lines.

Pollen tube growth was very sensitive to and dependent on the surrounding environment. Under in vitro conditions, pollen tubes in sensitive cultivars/lines grew slowly and often became distorted around tips within a few minutes of incubation at low temperatures while those in tolerant lines, ICCVs 88502 and 88503, continued to grow for a longer time without any apparent adverse effects (Table 3). The density and quality of neighbouring grains are considered

important under in vitro conditions (Brewbaker and Kwack, 1963), while the condition of the pistil is important for in vivo growth. Both stylar tissue and stigma exudates are known to modify pollen tube growth in chickpea (Turano et al., 1983; Malti and Shivanna, 1985). Pollen tubes growing in vitro are known to take up carbohydrates and amino acids (Kendall et al., 1971) and presumably, these and other nutrients are supplied by the style. The reduced pollen germination and slow or distorted pollen tube growth on the stigma could be due to the low amounts of exudates on the stigma and decreased supply of reserves by the style. The esterase test detects stigmatic surface proteins, which are known to be functionally important in retention and hydration of pollen grains (Turano et al., 1983). The low esterase activity in sensitive cultivars/lines suggests that stigmas in them were less receptive even if pollen were viable and germinable under optimum conditions. Indeed the amount of pollen picked up by the stigma in artificial pollinations was considerably less in sensitive cultivars/lines in the 15/0°C regime than in other regimes (data not shown). This may again be due to reduction at low temperature in the number of specialized papillate cells which produce a localized secretion (Turano et al., 1983).

Pollen tube growth in vivo was less in sensitive than in tolerant cultivars/lines. This was probably related to (a) insufficient food reserves accumulated in pollen grains and (b) low rates of basic metabolic activities (energy production, starch synthesis, wall binding, etc.) in pollen of sensitive cultivars/lines, the variability of which is likely to be controlled by genes equally expressed in sporophytic and gametophytic tissues (Ottaviano et al., 1982). Besides, there may be physical or physiological limitations operating at low temperature (e.g. the constriction of the lumen at the base of the style or a decrease in the size of transmitting tissue at the top of the style) that limit the growth of greater numbers of pollen tubes towards the ovary (Winsor, 1988). Reduced pollen tube growth in vivo at low temperature was also reported in snap bean (*Phaseolus vulgaris L.*) (Dickson and Boettger, 1984) and pear (*Pyrus domestica*) (Vasilakakis and Porlingis, 1985).

The resource status of a flower, which in turn is influenced by its location in sun or shade, position within the inflorescence, resource demands of other flowers, etc., is also probably important. In sensitive cultivars/lines, pollen tubes can rapidly exhaust the limited stylar resources which, in turn, may decrease the proportion of pollen tubes reaching the ovules. However, the cold-induced floral abscission could not be overcome even after extreme defloration indicating that poor pod set at low temperature may not be entirely due to competition for carbohydrates (Srinivasan, A., unpublished results). The existence of localized carbohydrate starvation in flowers developing at unfavourable temperature, as reported for cowpea (*Vigna unguiculata (L.) Walp.*) (Ahmed et al., 1993), cannot however, be ruled out.

The adverse effects of low day-time temperatures (15/5 vs. 20/5°C; 15/0 vs. 20/0°C) on pod and seed set as reported earlier (Srinivasan et al., 1998) may also be due to the negative influence of day temperature on pollen function. Dickson and Boettger (1984) reported that low night temperature was less important in bean (*Phaseolus vulgaris L.*) when the following day was also cool ($\leq$20°C), as pollen would grow too slowly to complete fertilization leading to reduced pod and seed set.

Ovule viability is another major component of pistil function besides stigma receptivity (Dumas et al., 1984). The high callose deposition (reduced viability) and low rates of fertilization of ovules in the 15/0°C regime in most cultivar/lines confirm that low temperature adversely affects pistil function. This is perhaps a major reason for lack of improvement in pod set of cultivars/lines such as Annigeri in cool regimes even when pollen from the control regime was supplied. The reduced ovule fertilization may also be due to the direct effects of low temperature on ovule maturity and embryo abortion. At the time of flower opening, ovules in sensitive cultivars/lines differed morphologically (size) and physiologically (viability) and were possibly at different developmental stages. Wilms (1981) reported that in spinach (*Spinicia oleracea* cv. Previtral) immature ovules cannot be penetrated by pollen tubes but in mature ovules, subsequent to pollination, synergids release substances which dissolve the middle lamellae of the nucellus in the micropyle region thus allowing pollen tubes to penetrate the ovule. Casper (1990) reported that in *Cryptantha flava*, most irreversible embryo abortion occurred during the establishment of the ovule size differences, and within a 24–48 h period. Even in

those instances where more than 10 pollen tubes were present at the base of the style, fertilization of all ovules in an ovary did not occur in sensitive cultivars/lines. In some cases, pollen tubes were present but they grew past unfertilized ovules.

Whether the male or female gamete was affected more severely by cold stress depended on cultivars/lines and severity of stress. In the 15/5°C regime, pollen was more adversely affected than pistil in ICCV 88510 and Pant G 114 while both male and female gametes were affected in Annigeri. Similar variation in sensitivity of male and female gametophytes to temperature was reported in maize (*Zea mays L.*) (Mitchell and Petolino, 1988) and tomato (*Lycopersicum esculentum L.*) (Levy et al., 1978). In the 15/0°C regime, however, both male and female gametes were adversely affected in all cultivars/lines tested here.

Pod set in sensitive cultivars/lines in the 15/5°C regime was markedly influenced by the source of pollen. The ability of pollen from sensitive cultivars/lines in 15/5 and 15/0°C regimes to bring about some pod set in the warm regime showed that pollen function response could be modified, at least partly, if favourable thermal regimes prevail after pollination. This result has an important implication because cold spells can be interspersed with warm periods under field conditions. In such cases, the ability of pollen to germinate and fertilize ovules can lead to pod set even in sensitive cultivars/lines. Further, because of a greater reduction in pollen function than pistil viability at moderately low temperatures (15/5°C) in many genotypes, the need for selection of germplasm with a greater degree of pollen function seems to be desirable at least during the initial stages of breeding.

The observation that occurrence of ovule fertilization did not always result in seed set suggested that early events in seed development were also sensitive to cold stress. These results confirmed the earlier findings (Srinivasan et al., 1998) that the threshold temperature for seed set was higher than for pod set. Farlow et al. (1979) reported similar differential temperature requirements for various processes of gametophyte function in bean (*Phaseolus vulgaris L.*). As most sensitive stages are during pollen–stigma interaction and seed set, the development of highly cold-tolerant lines would, however, require reduced sensitivity not only during pollen shed and stigma receptivity but also during pollen function and early seed development.

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