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# Evaluation of maize (*Zea mays*) inbred lines for tolerance to low temperature stress under field conditions

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ABSTRACT

In northern India, cold spell is experienced at early vegetative stage. Tolerance to cold in inbred lines is important to ensure seed production in *rabi* season. Sixty-six inbred lines of Indian maize program were evaluated for their tolerance to low temperature stress under field conditions. Maize (*Zea mays* L.) inbred lines of Bajaura Centre (BJIM 2780, BJIM 08-100, BJIM 10-1 and BJIM 10-35) and Almora Centre (V 366, V 384, V 351, V 382 and V 378) performed better with minimum leaf yellowing and good plant growth under the cold stress. Inbreds lines BJIM 10-35, BJIM 10-43, BJIM 10-37, V 364, V 404, V 390, LM 14 and BML 6 exhibited high reduction in yellowing of leaves coupled with fast growth recovery. Expression of yellowing of leaves and plant growth showed significant negative correlation. Yellowing of leaves and recovery of plant growth due to cold spell were important parameters for cold tolerance at early vegetative stage in maize.

**Key words:** Cold tolerance, Evaluation, Inbreds, *Zea mays*

Maize (*Zea mays* L.) is considered a chilling-sensitive species with a relatively high temperature optimum for germination, development and dry matter accumulation (Miedema 1982). Under climates characterized by cool and humid springs, adaptation has been partially successful due to late planting and breeding for early-maturing maize hybrids. These strategies are useful to minimize the risk of field losses due to chilling stress (Stamp 1986). Improvement of chilling tolerance would support earlier spring planting and, consequently, lead to higher yielding maize hybrids (Lee *et al.* 2002). Furthermore, earlier soil coverage would help to reduce erosive processes. At approximately the three-leaf stage of maize, seed reserves are exhausted and the seedling has to rely on its photosynthetic activity for carbon gain (Cooper and MacDonald 1970).

Cold-stress tolerance is a prerequisite for maize production under cool climatic conditions. In northern India, winter maize is grown in the month of October and November. During winter season maize crop is exposed to cold stress in the month of January. This stress may affect young plants after emergence. The inbred lines used as parental components when producing hybrid maize seed are particularly sensitive to cold stress. Nevertheless, if the inbred lines have sufficient genetic cold stress tolerance, seed producers endeavour to sow the seed as early as possible in order to make good use of available vegetation period and, perhaps more importantly, to ensure the flowering takes place before the plants are exposed to heat stress.

Low temperature prolongs growth duration, reduces crop growth rate, and thus weakens the seedling; however the duration of the winter crop of maize increases as we move towards northern parts. The chilling-sensitive nature of maize makes early plant establishment in spring difficult under cool environmental conditions (Jompuk *et al*. 2005). Among the various effects of low temperature on the physiology of maize, that on the photosynthetic apparatus is considered to be especially important (Baker *et al.* 1994). High chilling tolerance during autotrophic growth is accompanied by a high relative growth rate, sustained a high net assimilation rate in spite of a low leaf area ratio. Thus, the emphasis must be placed on photosynthesis. To avoid misinterpretation of the results, it is necessary to define the types of chilling stress. In maize, cold stress is known to reduce leaf size, stem extension and and root proliferation, disturb plant-water relations and impede water uptake (Farooq *et al.* 2009). The effects of low growth temperature below (15°C) on the photosynthetic apparatus of maize were studied by Fracheboud *et al*. (2002). They have reported a total of eight genomic regions which were significantly involved in the expression of target traits.The main objective of this study was to evaluate the Indian maize inbred lines for cold tolerance during the winter season in field conditions.

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## MATERIALS AND METHODS

A total of 66 inbred lines were used in the present study, which include 34 inbreds (extra-early/early maturing) from VPKAS, Almora, 27 inbreds (early/medium maturing) from Bajaura, 2 inbreds (late maturing) from PAU, Ludhiana and 3 inbred lines (late maturing) from ANGRAU, Hyderabad. The experiment was conducted during *rabi* season, 2010-11 at Directorate of Maize Research, Pusa Campus, New Delhi. Soil type of the experimental field is clayey loam alluvial type with a pH value of 7.2. All the lines were tested for cold tolerance in four replications randomized block design (RBD). Climate data recorded during experimental period showed that minimum temperature was less than 10°C for over one month during January, and occasionally dropped down below to 3°C. The inbred lines were sown on 24 November 2010. Experiments were kept free from insect, weeds and diseases using recommended agronomic practices. Each inbred line was planted in one row plot, each 2.0 m long, with 10 cm spacing within and 75 cm between rows. Before planting 60 kg nitrogen/ha in the form of urea, 60 kg phosphorous/ ha as single super phosphate, 40 kg potassium/ha as muriate of potash and 10 kg zinc as zinc sulfate were applied as a basal dressing. Second and third doses of N (each 30 kg N/ ha) were side-dressed at knee-high and tassel emergence stages. Pre-emergence application of atrazine (at 0.75 kg/ ha a.i., tank mixed) were used for weed management in the experimental plots.

Observations were recorded in field for yellowing of leaves, drying of leaves and level of growth (1 to 9 scale) at two stages, i e 18 January 2011 and 31 January 2011. The first date of observations was immediately after severe cold while the second date was after the temperature has slightly increased to observe the recovery in the maize plants. In addition, the data was also recorded for plant stand before the cold spell, after the cold spell and days to silk. The data on yellowing and drying of leaves was recorded on scale 1 (no yellowing/drying) to scale 9 (high yellowing/drying) as recorded by Mahajan *et al.* (2012). Similarly, the plant growth was recorded on scale 1 (poor plant growth) to scale 9 (excellent plant growth).Correlation studies were computed following Snedecor and Cochran (1967).

## RESULTS AND DISCUSSION

Changes in temperature, sunshine hours and average wind velocity were recorded throughout the cold spell during the month of January (data provided by Meteorological Division, IARI). The average minimum temperature during the first fortnight was 5.1°C, while during second fortnight it was 6.6°C. The minimum temperature for first fortnight was recorded <5°C continuously for ten days, while it was recorded only for five days during the second fortnight. Comparatively, much difference was observed between the average maximum temperature during the first fortnight (10°C) and the second fortnight (17°C).

The average minimum temperature during the first fortnight coupled with low sunshine hours (2.3 hr) and higher wind velocity (5.0 km/hr.) may have cumulative effect on the susceptibility of plants to cold (Mahajan *et al.* 2012). The optimal temperature for root, shoot and leaf elongation in maize is 30 to 35°C; the minimum temperature at which growth is completely inhibited is between 6 and 8°C (Miedema 1982).

During first fortnight, temperature and sunshine hours were observed lower in comparison with second fortnight. As the second fortnight progresses, the environmental conditions became slightly warmer and plants showed recovery from the cold symptoms with better growth.During the second fortnight, there was an increase in average temperature (11.7°C), sunshine hours (5.21) and decrease in the wind velocity (4.15).

All the traits like leaf colour, plant growth were significantly affected by the cold stress. Response of inbreds to cold was expressed by yellowing and initiation of drying of leaves during first recording of data. Reduced chlorophyll content in maize leaves under cold stress has also been reported by others (Leipner *et al.* 1999, Lee *et al.* 2002). Leaf yellowing showed significant variation among inbred lines under cold stress, which was more pronounced when best and worst type of lines were compared. Maize lines capable of accumulating high amounts of anthocyanin in the illuminated leaf surface may present an adaptive response to harmful conditions of low temperature associated with high light and it is not limiting to photosynthesis (Pietrini *et al.* 2002). In some cases the drying effect was so intense

Table 1 Performance of superior maize inbred lines for tolerance to cold expressed as yellowing of leaves (£4 on 1-9 scale) at stage I

| Inbred lines | Emergence (%) | Leaf yellowing (I) | Leaf yellowing (II) | Plant growth (I) | Plant growth (II) |
|---|---|---|---|---|---|
| BJIM 10-34 | 86.67 | 1.00 | 1.33 | 7.00 | 7.00 |
| V 366 | 76.67 | 1.33 | 1.33 | 5.67 | 6.33 |
| BJIM 2780 | 73.33 | 1.33 | 1.00 | 4.67 | 5.33 |
| BJIM 08-100 | 90.00 | 1.50 | 1.67 | 6.00 | 6.67 |
| V 384 | 96.67 | 1.67 | 1.00 | 5.67 | 6.00 |
| BJIM 10-1 | 83.33 | 2.50 | 1.67 | 7.00 | 7.33 |
| BJIM 10-1 | 83.30 | 2.50 | 1.67 | 7.00 | 7.33 |
| V 351 | 96.67 | 2.67 | 2.50 | 6.00 | 6.00 |
| BJIM 10-35 | 96.67 | 2.67 | 1.00 | 7.00 | 7.67 |
| V 382 | 80.00 | 3.00 | 2.67 | 5.33 | 6.00 |
| V 378 | 93.33 | 3.00 | 2.67 | 7.00 | 6.67 |
| BJIM 08-1 | 76.70 | 3.00 | 2.33 | 6.00 | 6.33 |
| V 335 | 83.30 | 3.67 | 3.33 | 4.33 | 5.33 |
| V 370 | 63.30 | 3.80 | 3.33 | 4.33 | 5.33 |
| V 364 | 86.70 | 4.00 | 2.67 | 5.67 | 5.33 |
| Range | (6330-96.67) | (1.00-4.00) | (1.00-3.33) | (4.33-7.00) | (5.33-7.67) |
| Average | 84.44 | 2.51 | 2.01 | 5.91 | 6.31 |
| SE± | 2.47 | 0.25 | 0.21 | 0.25 | 0.20 |

I- Stage I: immediately after cold spell, II- Stage II: after temperature started increasing that plants completely dried and could not recover even when the climate conditions became better during the second fortnight of the month.

Inbred lines V 366 (1.33), V 384 (1.67), V 351 (2.67), V 382 (3.00) and V 378 (3.00) of Almora Centre and BJIM 10-34 (1.00), BJIM 2780 (1.33), BJIM 08-100 (1.50), BJIM 10-1 (2.50) and BJIM 10-35 (2.67) of Bajaura Centre exhibited low score for yellowing and best score for plant growth at both the crop stages (Table 1). As soon as the climate conditions became warmer during the 2nd fortnight of January, these lines showed recovery of plant growth, i e the leaves become green and plants showed faster growth.

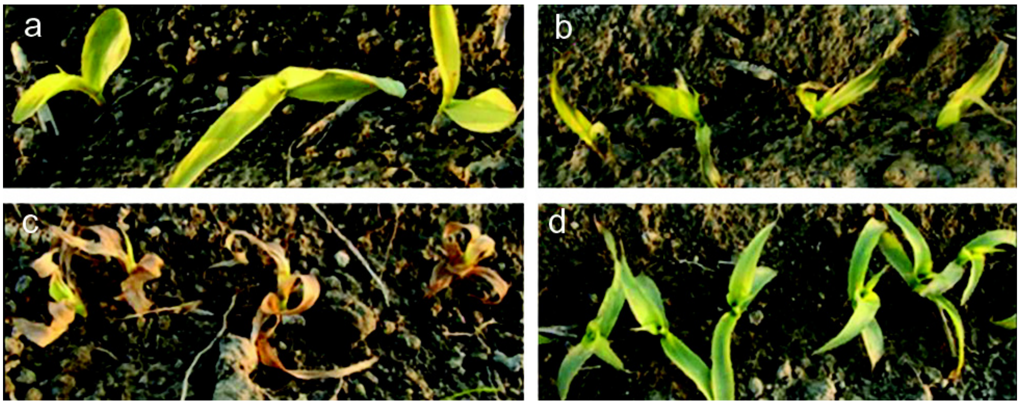


Fig 1 Inbred lines showing different cold symptoms, a- yellowing of leaves, b- drying initiation, c- completely dried plants and d- cold tolerant line

Collectively these lines were the best performing lines, i e least affected under the cold stress. The score for leaf yellowing was also low mostly in the lines which belong to the exta-early and early group comparatively with early flowering even in the winter season. These inbred lines may be used for development of cold tolerant hybrids with earliness. Mahajan *et al.* (2012) also reported exta-early and early maturing maize hybrids more tolerant to the cold temperatures. Similarly, most of the inbreds of Bajaura centre showed low score for leaf yellowing and good plant growth and will be promising for growing in winter season in north India.

Inbreds susceptible to the cold initially showed yellowing of leaves (leaves become paler) followed by drying of leaves. The drying was observed initially on leaf tips from where it progresses towards the main shoot resulting in complete drying of whole plant. Apart from visual score on leaf yellowing, variation for the developmental traits, such as plant growth was highly affected by the cold stress. This is in agreement with the observations made by Lee *et al.* (2002) on maize inbred lines, where they found that leaf appearance rate was about three times slow in cold stressed maize seedling (15/3°C; 16-h photoperiod) than grown under normal temperature (25/15°C; 16-h photoperiod). Leaf area growth was also reported to be a function of prevailing temperature regime. Tollenaar *et al.* (1979) reported that the optimum temperature for leaf extension in maize was 30°C, and the extrapolated minimum 7°C. Miedema (1982) also found genetic variation in leaf extension rate at day/night temperatures of 15/10,

Table 2 Recovery (%) of inbreds on the basis of leaf yellowing and plant growth

| Inbred lines | Leaf yellowing (I) | Leaf yellowing (II) | Reduction in yellowing (%) | Inbred lines | Plant growth (I) | Plant growth (II) | Recovery in plant growth (%) |
|---|---|---|---|---|---|---|---|
| BJIM 10-35* | 2.67 | 1.00 | –62.55 | Lines | 4.33 | 5.67 | 30.95 |
| BJIM 10-41 | 6.00 | 4.33 | –27.83 | V 374* | 5.33 | 6.33 | 18.76 |
| BJIM 10-38-1 | 5.67 | 4.33 | –23.63 | BJIM 08-100 | 6 | 6.67 | 11.17 |
| V 364 | 4.00 | 2.67 | –33.25 | V 382 | 5.33 | 6 | 12.57 |
| BJIM 08-105 | 6.00 | 4.67 | –22.17 | BJIM 10-43* | 4.67 | 5.33 | 14.13 |
| BJIM 10-43 | 5.33 | 4.00 | –24.95 | BJIM 10-35* | 7 | 7.67 | 9.57 |
| BJIM 10-33 | 6.33 | 5.00 | –21.01 | BJIM 08-202 | 3 | 3.67 | 22.33 |
| BJIM 10-32 | 5.00 | 4.00 | –20.00 | V 408 | 4 | 4.67 | 16.75 |
| V 400 | 5.00 | 4.00 | –20.00 | V 398 | 3.67 | 4.33 | 17.98 |
| V 338 | 5.67 | 4.67 | –17.64 | V 404* | 5.33 | 6 | 12.57 |
| V 404* | 5.00 | 4.00 | –20.00 | LM 14 | 4 | 4.67 | 16.75 |
| BJIM 10-32 | 5.67 | 4.67 | –17.64 | BJIM 08-27 | 3.67 | 4.33 | 17.98 |
| V 374* | 4.33 | 3.33 | –23.09 | V 366 | 5.67 | 6.33 | 11.64 |
| V 390* | 4.33 | 3.33 | –23.09 | BJIM 2780 | 4.67 | 5.33 | 14.13 |
| BML 6 | 4.33 | 3.33 | –23.09 | V 390* | 4.67 | 5.33 | 14.13 |
| Average | 5.02 | 3.82 | –25.33 | Average | 4.76 | 5.49 | 16.09 |
| SE± | 0.25 | 0.26 | 2.85 | SE± | 0.27 | 0.27 | 1.38 |

*Lines showing maximum reduction in yellowing of leaves coupled with fast plant growth recovery

Table 3 Association among emergence (%), leaf yellowing, plant growth and days to silk

| | Yellowing of leaves (I) | Plant growth (I) | Yellowing of leaves (II) | Plant growth (II) | Days to silk |
|---|---|---|---|---|---|
| Emergence (%) | –0.397* | 0.590** | –0.421** | 0.576** | –0.502** |
| Yellowness (I) | | –0.801** | 0.974** | –0.852** | 0.371* |
| Growth (I) | | | –0.824** | 0.969** | –0.519** |
| Yellowness (II) | | | | –0.874** | 0.354* |
| Growth (II) | | | | | –0.448** |

*P≤0.05;**P≤0.1

20/15, and 25/20°C.

Leaf yellowing ranged from 1 to 9 with maximum in BJIM 08-2(9.00), BJIM 10-44(9.00) and V 392(9.00), while minimum in BJIM 10-34(1.00), V 366 (1.33) and BJIM 2780(1.33). Inbred lines like V 368, V 345, V 394, V 410, V 391 and V 392 of Almora centre, BJIM 08-2, BJIM 10-44 of Bajoura centre, LM 13 of Ludhiana and BML 13 of Hyderabad exhibited high score for leaf yellowing (>7) and low score for plant growth (<3). Leaf drying was also observed in these lines. As the cold spell progresses, drying of plants became more and more severe. These lines do not show any recovery resulting in complete drying of plants even after the climate conditions became warmer during the second fortnight of the month.

The first recording indicated the expression of cold response of inbred lines, while the second recording showed the recovery of plant growth as well as reduction in yellowing of leaves. Maximum reduction in leaf yellowing due to the initiation of new leaves as well as faster plant growth was observed mostly in early and medium maturing inbred lines during the second fortnight of the month. Among the lines showing faster reduction in yellowing of leaves were BJIM 10-35, BJIM 10-41, BJIM 10-38-1, V 364, BJIM 08-105, V 364, BJIM 08-105, BJIM 10-43 and BJIM 10-33, while the inbreds showing faster plant growth were BJIM 08-103, V 374, BJIM 08-100, V 382, BJIM 10-37, BJIM 10-35 and BJIM 08-202. Inbreds showing maximum reduction in yellowing of leaves and faster plant growth were also observed (Table 2).

Correlation studies indicated that there is significantly high association among yellowing of leaves and plant growth at both the stages (Table 3). It was observed that leaf yellowing at stage I had strong and negative association with plant growth at both the stages (–0.801** and –0.852**, respectively). Correlation studies demonstrated that neither yellowing of leaves (0.37*) nor plant growth (0.35*) were strongly associated with days to silk. Similar results have been reported for two maize populations adapted to the Central US Corn Belt (Mock and Eberhart 1972) and 144 plant introductions of maize (Mock and Skrdla 1978).

Inbred lines of all the maturity groups are suitable for sowing in the Rabi season. The yellowing of leaves is an important criterion and had high correlation with the recovery of the plant which is also expressed in yield.

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