Cold Stress Evaluation among Maize (*Zea mays* L.) Inbred Lines in Different Temperature Conditions

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**ABSTRACT** Maize (*Zea mays* L.) is a crop in a tropical region which resists growing under sensitive temperature. This study was conducted to evaluate the performance of Canadian maize inbred lines under controlled cold stress conditions (5°C, 10°C, and 23°C). Data were recorded by measuring germination rate, index, root length, and seed vigour index values. Five higher and three lower tolerant inbred lines were shortlisted. The data were analyzed using analysis of variance, while mean values were compared using Tukey’s Honest Significant Difference Test at \(\alpha=0.05\) and at \(\alpha=0.01\). Using Genstat software, correlation was done. A strong correlation \((P<0.05)\) was found between germination rate and germination index under all stress conditions. Root length and vigour index were also strongly correlated with germination rate under 5°C stress condition and compared to 10°C and 23°C stress conditions. Our results suggested that five (CO439, CO438, CO450, CO435, and CO445) among 22 maize inbred lines performed better under 5°C cold stress condition and thus had the potential to develop maize hybrids to increase grain yield under environmentally stressful conditions in South Korea.

**Keywords** Cold tolerance, Germination, Vigour, Maize, Inbred line

**INTRODUCTION**

Crop plants respond to environmental changes by modifying their pattern of gene expression and products under stressful conditions for survival and yield (Sanghera *et al.* 2011). Mirosvavljević *et al.* (2013) found that root weight, shoot weight, root length (RL), shoot length, germination energy, and rate were affected due to the stress in plants. Hence, tolerance of crop plants is essential for vigourous growth and for resisting insects and pests. Thus, for good yield and to withstand insects and pests, crops should be resistant against various abiotic and biotic factors. Chilling temperature is one of the important constraints during early seedling growth for global crop (maize) production (Guan *et al.* 2009; Ali *et al.* 2015a). Cold stress can influence periodic life cycle and productivity of crop plant in the early developmental stages. Chilling stress basically disturbs mean germination time (MGT) among cultivars; this is significantly correlated with the shoot length and dry weight of plant. Furthermore, low temperature affects photosynthetic rate, secondary osmotic, and oxidative stress which lead to cell damage (Matthews *et al.* 2011; Riva-Roveda and Périlleux 2015).

Maize (*Zea mays* L.) is highly sensitive to low temperature due to their tropical origin. Abiotic stresses cause adverse effect in growth, physiology, and biochemical processes (Ali *et al.* 2015b). Nowadays, maize crop is cultivated in a wide range of altitudes. Many adaptations have been implemented to keep its high production yield (Ali *et al.* 2014). In maize plants, cold stress germination traits are important to check for earlier seasonal adaptability (Hoffman *et al.* 2015). Revilla *et al.* (2014) revealed that the cold tolerance of maize is an important investigation factor for the vegetative period extension. Matthews *et al.* (2011) suggested that, compared with other germination tests, cold test is better in assessing early...
growth vigour. Among the maize inbred lines, there is great variability for the cold tolerance evaluation under certain environmental conditions. Germination under any kind of stress is important to assume the vigour of that plant (Alvarez et al. 2014). Cold tolerance is a complex study to investigate; however, molecular biology and physiological approaches are the best ways to evaluate the low temperature response of maize at sprouting stage (Bano et al. 2015). Seedling vigour and RL are directly influenced with environmental stress.

Furthermore, seed storage is a big issue under various climatic conditions. For seed storage to ensure quality, lower temperature is better than high temperature. Thus, evaluation under cold stress is essential. Chilling temperature halts membrane permeability, photosynthetic rate, and osmotic potential in plant; at high temperature conditions, seed deterioration increases due to high moisture acceleration (Abba and Lovato 1999). In cold tolerance, multiple genes are involved to control chilling stress. The quality of maize seed in chilling temperature can be negatively affected. However, the genotype with compact root system has good metabolic activities and ability to resist against harsh conditions. It can sprout better seedling and more vigour (DeVries et al. 2007). The cold storage of seed prevents pest/insect attack and helps seeds resist microorganisms. Seed quality becomes affected when moisture content is higher than 14% (Govender et al. 2008). At low temperature, plants respond to alterations in the expression of gene and protein product pattern. Genes can figure out, through germination testing, which are responsible for stress response during the germination of crop plant.

At cold temperature, physiological damage can reduce seed vigour and viability (DeVries et al. 2007). However, different genotypes have different levels of survival under cold environment as their genetic makeup can either endure or is not tolerant against freezing temperature. The best evaluation of maize germination can be done when a plant shows rapid germination and vigour, and resists insect pests under low temperature stress. The seed vigour and viability loss are greater in decreasing temperature or abrupt changes in environmental conditions (TeKrony and Hunter 1994; Woltz et al. 2006). Tekrony (2003) revealed from his experimentation that seed vigour test is better for the evaluation of physiological seed quality. Crop yield reduction is indirectly related with low seed vigour. During cold germination, the significant difference values of root and shoot among different genotypes were exhibited.

Hence, seed vigour and viability directly impact the crop yield and performance of seed quality under certain conditions. The increase or decrease of yield in crop plants is directly associated with seed vigour. Thus, germination test is necessary to check the performance of different cultivars before cultivation. This study’s results demonstrate that study maize inbred lines have the potential to develop maize hybrids in cold climatic condition such as in South Korea. In detail, our aims to conduct this experiment were:

i) To identify the seed vigour, germination, and seedling growth of maize inbred lines in different temperature conditions.

ii) To check early germination and establishment associated with important physiological and phonological characters.

iii) To screen out the diversified pool of maize germplasm that can tolerate chilling temperature and differentiate high and low tolerant inbred lines, which will be further experimented in the molecular breeding program.

MATERIALS AND METHODS

Seed material

A total of 22 maize inbred lines, developed at the Institute of Eastern Cereal and Oilseed Research Centre in Canada, were used in this study (Table 1). Seeds for each inbred line were soaked in water before going to a germination test. All seeds were treated with Captan (cis-N trichloromethylthio-4-cyclohexene-1, 2-dicarboximide).

Cold germination test

Testing was carried out in a growth chamber in three replicates with 20 seeds of each inbred line per replicate. Seeds were planted on petri dishes with moist filter paper and placed in growth chambers at constants of 5°C, 10°C,
and 23°C under completely randomized design. Five ml of distilled water was added at the beginning of the experiment and every two days during the duration of the study. The number of germinated seeds was recorded daily for 7 days. Seeds were considered germinated when the radicle was 2 mm long. Evaluations of germination were made every day. Seeds were tested in chilling temperature under International Seed Testing Association (ISTA) (2002) seed testing rules.

### Germination analysis

**Germination rate (GR):** The GR (%) of seeds of each inbred line was calculated with the appropriate formula:

\[
GR(\%) = \frac{\text{number of seeds sprouted}}{\text{total number of seeds sprouted}} \times 100
\]

**Germination index (GI):** GI and MGT were calculated using the following formula:

\[
GI = \sum \left( \frac{G_t}{T_t} \right), \text{ where } G_t \text{ is the number of seeds germinated on each day } (t), \text{ and } T_t \text{ is the total number of days for germination.}
\]

| Inbred/Autogame | Derivation/Source | Heterotic group |
|------------------|-------------------|-----------------|
| CO430            | Fusarium Resistant Synthetic | P3990          |
| CO431            | Fusarium Resistant Synthetic | Iodent         |
| CO432            | Fusarium Resistant Synthetic C1 | Minn13        |
| CO433            | Pride K127         | Minn13         |
| CO434            | CM105 × A632       | BSSS           |
| CO435            | A632 × A634        | BSSS           |
| CO436            | CO275 × CO300      | P3994          |
| CO437            | European Synthetic | E.Flint        |
| CO438            | CB3 × CL29         | P3994          |
| CO439            | Nebraska BSSS      | BSSS           |
| CO440            | Pride 5 × CO258V   | Minn13         |
| CO441            | Jacques 7700 × CO298 | Lanc         |
| CO442            | Iodent/NSS         | Iodent         |
| CO443            | B104 × CO272       | BSSS/E.Butler |
| CO444            | S1381 × CO382      | E.Flint        |
| CO445            | CO386 × W64AHt     | Lanc           |
| CO446            | CO341 × CO328      | BSSS           |
| CO447            | CO352 × CO328      | BSSS/Minn      |
| CO448            | CO273 × CO431      | P3990/Iodent   |
| CO449            | CO432 × CO433      | Minn13         |
| CO450            | Eyespot Resistant Synthetic (99ESR) | BSSS/Mix |
| CO451            | CO309 × CO328      | BSSS/Minn      |

MGT=\(\sum T_i N_i / \sum N_i\), where \(N_i\) is the number of newly germinated seeds at time \(T_i\) (Ruan et al. 2002).

**RL:** Root growth was evaluated by hand using a ruler in mm. All three temperature treatments were separately evaluated in the same set of seeds.

**Seed vigour index (SVI):** Using the formula of Abdul-Baki and Anderson (1973), SVI was calculated.

\[
SVI = \frac{\text{Seedling emergence rate} \times RL \text{ [mm]}}{100}
\]

### Statistical analysis

The statistical analysis was performed using Microsoft Excel (2007; Microsoft, Redmond, WA, USA) and Statistical Tools for Agricultural Research (STAR), while analysis of variance (ANOVA) was conducted using SAS 9.3 software (SAS Institute, Cary, NC, USA). Means were compared using Tukey’s Honest Significant Difference (HSD) Test at \(\alpha=0.05\) and at \(\alpha=0.01\). The correlation analysis was done using statistical software (Genstat; VSN International, Hemel Hempstead, UK).
RESULTS

Effect of germination status in different temperature conditions

A total of 22 maize inbred lines were investigated at three different temperatures (5°C, 10°C, and 23°C, respectively). The traits were compared with least significant difference values. At lowest temperature (5°C) treatment, the highest rate of germination was shown in CO439 inbred line. The CO451 and CO450 inbred lines showed the highest GR at 10°C treatment. Almost, all inbred lines showed higher GR at 23°C condition, which was almost near room temperature. In variation, the GR of CO450, CO435, CO444, and CO451 were relatively higher at 10°C as compared to 23°C (Table 2). As a result, four inbred lines, namely CO439, CO438, CO450, CO435, and CO445 (weaker only at 10°C), exhibited a high GR of 70% at different temperatures (5°C, 10°C, and 23°C). Whereas, three inbred lines, namely CO437, CO436, and CO440, showed the lowest GR under the above given germination parameters in the control condition. The rest of the maize inbred lines showed medium GR, GI, RL, and SVI.

At lowest temperature (5°C) treatment, the highest and lowest values of GI were observed in CO438 and CO440, respectively (Table 3). At 10°C, CO450 showed the highest value of GI, and CO436 showed the lowest value of GI. At 23°C, most inbred lines showed comparatively high GI, except for two inbred lines (CO443 and CO437). The longest RL was observed in CO433, and the shortest RL was observed in CO440 at 5°C treatment (Table 4). At 10°C, CO444 showed the longest RL, and CO436 showed the shortest RL. At 23°C, CO441 showed the longest RL, and CO436 showed the shortest RL.

In addition, Table 5 demonstrates the SVI data at different temperatures of different inbred lines. The strongest SVI was observed in CO439, and the weakest SVI was

Table 2. Germination rate of 22 maize inbred lines in different temperature conditions.

| Inbred lines | Germination rate (%) at different temperature conditions | Characters |
|--------------|----------------------------------------------------------|------------|
|              | 5°C | 10°C | 23°C |                          |
| CO439        | 90.0a<sup>2)</sup> | 98.3a | 98.3a | High tolerant |
| CO438        | 88.3a | 81.6abc | 90.0abc | High tolerant |
| CO450        | 85.0ab | 100.0a | 98.3a | High tolerant |
| CO435        | 80.0abc | 98.3a | 91.6abc | High tolerant |
| CO445        | 75.0abcd | 45.0ef | 91.6abc | High tolerant |
| CO444        | 65.0bcde | 95.0a | 98.3a | Medium tolerant |
| CO433        | 65.0bcde | 90.0ab | 93.3ab | Medium tolerant |
| CO447        | 61.6cde | 76.6abc | 96.6ab | Medium tolerant |
| CO431        | 60.0cdef | 86.6ab | 90.0abc | Medium tolerant |
| CO451        | 55.0defg | 100.0a | 90.0abc | Medium tolerant |
| CO442        | 53.3efgh | 65.0bcdef | 96.6ab | Medium tolerant |
| CO443        | 40.0fghi | 50.0cdef | 66.6e | Medium tolerant |
| CO449        | 36.6ghi | 88.3ab | 93.3ab | Medium tolerant |
| CO441        | 33.3hi | 40.0fg | 91.6abc | Medium tolerant |
| CO446        | 33.3hi | 43.3g | 100.0a | Medium tolerant |
| CO432        | 31.6i | 90.0ab | 91.6abc | Medium tolerant |
| CO434        | 30.0i | 88.3ab | 100.0a | Medium tolerant |
| CO430        | 23.3ij | 73.3abcde | 73.3de | Medium tolerant |
| CO448        | 23.3ij | 93.3ab | 88.3abc | Medium tolerant |
| CO437        | 8.3jk | 65.0bcdef | 80.0cd | Low tolerant |
| CO436        | 3.3jk | 11.6g | 85.0bcd | Low tolerant |
| CO440        | 1.6k | 55.0cdef | 93.3ab | Low tolerant |

<sup>2)</sup>Means in the same column followed by different letters are significantly different according to Tukey’s New Multiple Range test (P<0.05).
Table 3. Germination index of 22 maize inbred lines in different temperature conditions.

| Inbred lines | Germination index at different temperature conditions | Characters |
|--------------|------------------------------------------------------|------------|
|              | 5°C | 10°C | 23°C |                     |
| CO439        | 1.7a | 2.2ab | 7.4abcd | High tolerant |
| CO438        | 1.8a | 1.6bcde | 6.2ede | High tolerant |
| CO450        | 1.6a | 2.3a | 7.8abc | High tolerant |
| CO435        | 1.5ab | 2.0abc | 7.3abed | High tolerant |
| CO445        | 1.5abc | 1.0fg | 7.3abed | High tolerant |
| CO444        | 1.2bcd | 2.0abc | 6.6bcd | Medium tolerant |
| CO433        | 1.2bcd | 2.0abc | 7.4abcde | Medium tolerant |
| CO447        | 1.2bcd | 1.6bcde | 7.5abc | Medium tolerant |
| CO431        | 1.1cd | 1.7abce | 6.4cd | Medium tolerant |
| CO451        | 1.0def | 2.0abc | 7.0bcd | Medium tolerant |
| CO442        | 1.1de | 1.5cdef | 7.4bcde | Medium tolerant |
| CO443        | 0.7efg | 1.0fg | 4.7e | Medium tolerant |
| CO449        | 0.7efg | 1.8abced | 7.3abed | Medium tolerant |
| CO441        | 0.6fg | 0.8gh | 7.1abc | Medium tolerant |
| CO446        | 0.6fg | 1.0fg | 8.0ab | Medium tolerant |
| CO432        | 0.6fg | 2.0abc | 7.7abc | Medium tolerant |
| CO434        | 0.6g | 1.8abced | 8.7a | Medium tolerant |
| CO430        | 0.4gh | 1.5cdef | 7.0bcd | Medium tolerant |
| CO448        | 0.4gh | 2.0abc | 6.2cde | Medium tolerant |
| CO437        | 0.1hi | 1.3defg | 5.8de | Low tolerant |
| CO436        | 0.1hi | 0.3h | 6.5bcd | Low tolerant |
| CO440        | 0.03i | 1.2efg | 7.8abc | Low tolerant |

<sup>z</sup>Means in the same column followed by different letters are significantly different according to Tukey’s new multiple range test (<i>P</i>&lt;0.05).

Table 4. Root length of 22 maize inbred lines in different temperature conditions.

| Inbred lines | Root length (mm) at different temperature conditions | Characters |
|--------------|------------------------------------------------------|------------|
|              | 5°C | 10°C | 23°C |                     |
| CO439        | 83.6ab<sup>z</sup> | 55.0def | 54.3bcdefg | High tolerant |
| CO438        | 50.6def | 47.0defgh | 57.6abcede | High tolerant |
| CO450        | 56.0cdef | 75.3abc | 60.3bcdef | High tolerant |
| CO435        | 71.6bcd | 46.0defgh | 67.0abc | High tolerant |
| CO445        | 41.3efghi | 51.0defg | 67.0abc | High tolerant |
| CO444        | 86.0ab | 81.0a | 63.3abcde | Medium tolerant |
| CO433        | 95.0a | 37.6fghi | 57.0abcdefg | Medium tolerant |
| CO447        | 58.0cdef | 57.6cd | 72.0ab | Medium tolerant |
| CO431        | 76.3abc | 51.3defg | 52.0cdefg | Medium tolerant |
| CO451        | 51.3def | 57.3cde | 50.0cdefg | Medium tolerant |
| CO442        | 65.0bcde | 63.6abc | 49.6cdefgh | Medium tolerant |
| CO443        | 36.6fghi | 57.0cde | 45.6efg | Medium tolerant |
| CO449        | 48.6defg | 61.0bced | 68.0abc | Medium tolerant |
| CO441        | 49.6def | 50.6defg | 73.6a | Medium tolerant |
| CO446        | 71.3bcd | 36.6fghi | 47.3efg | Medium tolerant |
| CO432        | 61.6bcd | 34.3ghi | 47.3efg | Medium tolerant |
| CO434        | 45.3efg | 29.0ghi | 42.0fg | Medium tolerant |
| CO430        | 49.6def | 79.0ab | 60.0abcdef | Medium tolerant |
| CO448        | 43.3efghi | 77.6ab | 49.6cdefgh | Medium tolerant |
| CO437        | 18.6hi | 56.6cde | 63.0abcde | Low tolerant |
| CO436        | 24.0ghi | 25.3i | 40.0g | Low tolerant |
| CO440        | 17.6i | 38.6fghi | 48.6defg | Low tolerant |

<sup>z</sup>Means in the same column followed by different letters are significantly different according to Tukey’s new multiple range test (<i>P</i>&lt;0.05).
Table 5. Seed vigour index of 22 maize inbred lines in different temperature conditions.

| Inbred lines | Seed vigour index at different temperature conditions | Characters |
|--------------|-----------------------------------------------------|------------|
|              | 5°C | 10°C | 23°C |                     |
| CO439        | 74.5a | 54.1abcd | 53.5abcdefg | High tolerant |
| CO438        | 44.8bede | 38.0bcdefg | 37.0gh | High tolerant |
| CO450        | 47.3bcde | 75.3a | 59.4bcde | High tolerant |
| CO435        | 57.8abc | 45.0bcdefgh | 36.9gh | High tolerant |
| CO445        | 31.4efghi | 23.2fgh | 62.2abcde | High tolerant |
| CO444        | 55.7abcd | 77.1a | 62.1abcde | Medium tolerant |
| CO433        | 61.9ab | 34.1cdefg | 52.2abcdefg | Medium tolerant |
| CO447        | 36.5defgh | 44.9bcdefg | 69.6a | Medium tolerant |
| CO431        | 45.6bcdef | 44.5bcdefg | 47.1defgh | Medium tolerant |
| CO451        | 27.6fghij | 57.3abc | 44.8efgh | Medium tolerant |
| CO442        | 39.5cdefg | 47.4bcdef | 48.0cdefgh | Medium tolerant |
| CO443        | 15.7ijk | 27.4efgh | 49.9bcdefgh | Medium tolerant |
| CO449        | 19.1hijk | 53.8abcd | 65.7abcd | Medium tolerant |
| CO441        | 16.3ijk | 19.7gh | 67.8ab | Medium tolerant |
| CO446        | 23.3ghij | 19.9gh | 47.3defgh | Medium tolerant |
| CO432        | 19.5hijk | 30.5defg | 44.2efgh | Medium tolerant |
| CO434        | 13.9ijk | 25.5fgh | 67.0abc | Medium tolerant |
| CO430        | 12.4ijk | 57.4abc | 44.0efgh | Medium tolerant |
| CO448        | 11.4ijk | 52.3abce | 55.8abcdefg | Medium tolerant |
| CO437        | 3.0k | 53.0abcd | 39.8fgh | Low tolerant |
| CO436        | 1.2k | 2.9h | 30.6h | Low tolerant |
| CO440        | 0.8k | 22.8fgh | 45.5efgh | Low tolerant |

Means in the same column followed by different letters are significantly different according to Tukey’s new multiple range test ($P<0.05$).

Table 6. Correlation coefficients of under-studied traits at 5°C, 10°C, 23°C temperature condition.

| Temperature | Traits$^\dagger$ | GR | GI | RL | SVI |
|-------------|----------------|----|----|----|----|
| At 5°C       | GR             | 0.90 |    | 0.60 | 0.80 | 0.81 |
|              | GI             |    | 0.60 | 0.64 |    |    |
|              | RL             |    | 0.80 | 0.81 | 0.81 |    |
| At 10°C      | GR             | 0.98 |    | 0.32 | 0.75 | 0.83 |
|              | GI             |    | 0.32 | 0.30 |    |    |
|              | RL             |    | 0.75 | 0.75 | 0.83 |    |
| At 23°C      | GR             | 0.81 |    | 0.25 | 0.56 | 0.93 |
|              | GI             |    | 0.25 | 0.18 |    |    |
|              | RL             |    | 0.56 | 0.45 | 0.93 |    |

$^\dagger$P-value=0.05.

observed in CO440 at 5°C treatment. At 10°C, CO444 showed the strongest SVI, and CO436 showed the weakest SVI. At 23°C, CO447 showed the highest value for SVI, and CO436 showed the weakest SVI. In our study, CO439, CO438, CO450, CO435, and CO445 showed the best response in all given traits, while CO437, CO436, and CO440 showed the weakest response accordingly.
Seed germination patterns in different temperature conditions

Supplementary Fig. 1 showed the distribution pattern for GR, GI, RL, and SVI investigated in 22 maize inbred lines under three temperature conditions (5°C, 10°C, and 23°C, respectively). A strong correlation was also found between GR and GI values under all temperature conditions (5°C, 10°C, and 23°C); that is, with the increase in GR values there was a notable increase in GI as shown in Table 6. The analysis showed that least significant difference values among all studied traits were highly significant (that means all of the traits tested differed from all other traits).

The GR of maize lines CO439, CO438, CO450, and CO435 was found highly significant under all temperature conditions (P < 0.05). The CO440 showed the lowest GR at 5°C, CO436 at 10°C, and CO443 at 23°C. Upon analyzing the GI of 22 maize inbred lines in different temperature conditions, the overall better performance was found in CO439, CO438, CO450, and CO435, while the lowest tolerant behavior was found to be the same for CO437, CO436, and CO440 inbred lines under all studied temperatures (Table 3).

When we look for the RL of 22 maize inbred lines in different temperature conditions, the performance was found significant for medium tolerant maize line (Table 4). The RL was found to be more significant with the GR under 5°C treatment. It was shown that under various temperature conditions, such as 5°C, 10°C, and 23°C, the different maize inbred lines CO433, CO444, and CO449 respectively showed significant mean values while no significant difference was found when analyzing the lowest tolerant lines as CO436 and CO440. For SVI, we found that it performed better under stress conditions 5°C (CO439), 10°C (CO444), and 23°C (CO445) (Table 5). The CO436 and CO440 showed the worst performance by giving the lowest significant mean values.

The overall performance of all the maize lines under different temperatures showed the significant behavior of better performing maize lines. SVI was also strongly correlated with GR under 5°C stress conditions. A similar finding was observed between GI and RL (strongly associated) under 5°C stress conditions (Table 6). The RL and SVI were strongly associated under all cold stress conditions (Table 6). Our correlation findings report that understudied lines performed better under 5°C cold stress as shown in Table 6, which clearly demonstrates that these lines have the potential to develop maize hybrids to increase grain yield under environmentally stressful conditions.

DISCUSSION

In many countries, maize crop has been adopted by sowing in early spring due to the low temperature of soil. Seeds can imbibe at chilling temperature, but often cannot germinate and may be attacked by fungi. In this study, we have observed the variance and significantly different values under three different cold temperature conditions. In results, some inbred lines were performed even at low compared with optimum temperature. It may be due to inbred lines at optimum temperature are more susceptible for disease. Table 2 demonstrates that the GR of seeds was mostly affected at all the studied temperature conditions. All lines gave higher rate because the mitochondria of plant cell are deeply affected due to low temperature during seedling growth. Furthermore, plants resist growing with an affected GR due to the seizing off of metabolic activities in plant cell (De Santis et al. 1999). Lovato et al. (2005) conducted a comparative experiment to check the relationship of seed emergence among germination tests. The standard cold temperature for maize is 10°C that may not be the favorable temperature in maize genotypes due to the hardness of cold temperature variance. Thus, they suggested that for the evaluation of cool tolerant genotypes, 5°C or 7.5°C is better to detect vigour difference among seed lots.

In a comparative result of GI, the values were the same as the GR from least significant different values in Table 3. This happened because the extremely low temperature reduced the germination and seedling growth as confirmed from the literature (Zheng et al. 2006). Wijewardana et al. (2015) also revealed similar results for cold tolerant corn hybrids. They reported that cold tolerant corn hybrids were useful to withstand harsh environmental conditions.
Maximum yield can be approached in early season seeding and is more beneficial for breeders, as well as growers. An alternative cold test for maize germination was conducted by Matthews and Khajeh-Hosseini (2007). They suggested from their findings that quality of seed has great influence on seed vigour. Germination in older seeds can be delayed due to longer lag periods allow in seeds to repair metabolic and physiological activities (Matthews et al. 2011). Thus, the vigour of seed and seedling growth are also affected.

In our cold test result, RL values vary among lines (Table 4) not only affected by temperature stress, but also by seed quality and other metabolic activities during root growth. Plant roots are directly associated with suitable environment. At chilling temperature during water uptake, metabolic and biochemical activities are affected as the nutrient supply photosynthetic activities in crop plant and carbohydrate supply to shoot are directly related with root synthesis (Hund et al. 2008). Genotypes with high vigour must have the ability for root growth under freezing temperature. Higher root and shoot length are directly associated with seedling dry weight. Imran et al. (2013) also reported plant germination with increased ability to grow root under cold condition. Aroca et al. (2001) found in some maize root that hydraulic conductance activity seized at low temperature.

The embryo of seed is directly associated with vigorous seedling. In cereals, large grain promotes early vigour (Revilla et al. 1999). Higher vigour of crop plant is directly associated with higher yield. SVI values from our experiment were also affected at the lowest temperature value of 5°C (Table 5). The more precise assessment of physical and physiological seed lot performance can be done through vigour test. Kollipara et al. (2002) conducted an experiment to screen out recombinant inbred lines for cold germination and desiccation tolerance of phenotypes. From the results, it became easy to understand the gene involved in stress response during seed maturity and germination.

Rodriguez et al. (2010) conducted a research on maize in temperate areas at cold temperature. Among 95 screened members of the population, 11 exhibited best germination vigour under cold condition. At low temperature, a plant cell’s metabolic, biochemical, and aerobic respiratory activities decrease. As a result, reactive oxidation species or plant growth retarding enzyme is produced, directly affecting plant germination and seedling vigour. However, for breeders and producers, the cultivar which shows the best performance against chilling temperature is highly recommended. To screen out stress tolerant cultivar is a challenging phenomenon in plant science. Thus, our findings will be helpful to carry out molecular design breeding in maize plant.

In conclusion, we can assume from the results of the cold germination test that at 5°C, 10°C, and 23°C, almost all 22 maize inbred lines show optimum germination and vigour. There is strong correlation between GR and index. In the overall comparison of studied traits, CO439, CO438, CO450, CO435, and CO445 show higher tolerance against all temperatures. Meanwhile, CO437, CO436, and CO440 lines perform weakly under the same circumstances. These selective high and low tolerant lines will be further investigated in molecular research and test crosses. This study also provides the basis for maize breeding research to assess best tolerant varieties under harsh conditions.

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