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). Mirosavljević *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 vigorous 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

Received August 12, 2016; Revised August 16, 2016; Accepted August 17, 2016; Published August 31, 2016

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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,

Table 1. Derivations of Canadian maize inbred lines used in this study.

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

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:

$$\text{GR (\%)} = (\text{number of seeds sprouted} / \text{total number of seeds}) \times 100$$

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

$$\text{GI} = \sum (\text{Gt} / \text{Tt}), \text{ where Gt is the number of seeds germinated on each day (t), and Tt is the total number of days for germination.}$$

$\text{MGT} = \sum \text{Ti Ni} / \sum \text{Ni}$, where Ni is the number of newly germinated seeds at time Ti (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.

$$\text{SVI} = (\text{Seedling emergence rate} \times \text{RL [mm]}) / 100$$

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 ²⁾	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	76.6abcd	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	50.0def	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

²⁾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⁾	2.2ab	7.4abcd	High tolerant
CO438	1.8a	1.6bcde	6.2cde	High tolerant
CO450	1.6a	2.3a	7.8abc	High tolerant
CO435	1.5ab	2.0abc	7.3abcd	High tolerant
CO445	1.5abc	1.0fg	7.3abcd	High tolerant
CO444	1.2bcd	2.0abc	6.6bcd	Medium tolerant
CO433	1.2bcd	2.0abc	7.4abcd	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	7.4abcd	Medium tolerant
CO443	0.7efg	1.0fg	4.7e	Medium tolerant
CO449	0.7efg	1.8abcd	7.3abcd	Medium tolerant
CO441	0.6fg	0.8gh	7.1abcd	Medium tolerant
CO446	0.6fg	1.0fg	8.0ab	Medium tolerant
CO432	0.6fg	2.0abc	7.7abc	Medium tolerant
CO434	0.6g	1.8abcde	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

²⁾Means in the same column followed by different letters are significantly different according to Tukey's new multiple range test ($P<0.05$).

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 ²⁾	55.0def	54.3bcdefg	High tolerant
CO438	50.6def	47.6defgh	57.6abcdeg	High tolerant
CO450	56.0cdef	75.3abc	60.3abcdef	High tolerant
CO435	71.6abcd	46.0defgh	67.0abcd	High tolerant
CO445	41.3efghi	51.0defg	67.0abcd	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	63.6abcd	49.6cdefgh	Medium tolerant
CO443	36.6fghi	57.0cde	45.6efg	Medium tolerant
CO449	48.6defg	61.0bcd	68.0abc	Medium tolerant
CO441	49.6def	50.6defg	73.6a	Medium tolerant
CO446	71.3abcd	36.6fghi	47.3efg	Medium tolerant
CO432	61.6bcde	34.3ghi	47.3efg	Medium tolerant
CO434	45.3efg	29.0hi	42.0fg	Medium tolerant
CO430	49.6def	79.0ab	60.0abcdef	Medium tolerant
CO448	43.3efgh	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	38.6efghi	48.6defg	Low tolerant

²⁾Means in the same column followed by different letters are significantly different according to Tukey's new multiple range test ($P<0.05$).

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 ²⁾	54.1abcd	53.5abcdefg	High tolerant
CO438	44.8bcde	38.0bcefg	37.0gh	High tolerant
CO450	47.3bcde	75.3a	59.4abcde	High tolerant
CO435	57.8abc	45.0bcefg	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	11.4jk	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

²⁾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 ²⁾	GR	GI	RL	SVI
At 5°C	GR				
	GI	0.90			
	RL	0.60	0.64		
	SVI	0.80	0.81	0.81	
At 10°C	GR				
	GI	0.98			
	RL	0.32	0.30		
	SVI	0.75	0.75	0.83	
At 23°C	GR				
	GI	0.81			
	RL	0.25	0.18		
	SVI	0.56	0.45	0.93	

²⁾ 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.

Rodríguez *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.

ACKNOWLEDGEMENTS

This study was supported by the Golden Seed Project (No. 213001-04-1-SBA10), the Ministry of Agriculture, Food, and Rural Affairs (MAFRA), the Ministry of Oceans and Fisheries (MOF), the Rural Development of Korea (RDA), and the Korea Forest Service (KFS).

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