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Assessment of cold tolerance in maize inbreds in North-Western Himalayas of Jammu and Kashmir, India

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ABSTRACT

Cold stress damage is one of the main meteorological disasters that can befall maize production, especially in northern regions and high-altitude areas in mountainous regions. This study was aimed to characterize a set of 164 maize inbreds for cold tolerance and identify the most promising maize inbreds for cold tolerance in the northwestern Himalayas of state Jammu and Kashmir, India at Mountain Crop Research Station, Larnoo, SKUAST-K, J&K, India. We investigated chilling-induced responses in 164 maize inbred lines through field-based evaluation utilizing the Cold Tolerance Rating (CTR) and controlled-environment assessment employing the electrolyte leakage assay. Inbreds were screened for cold tolerance using a rating scale of 1–5 and cell membrane stability under cold stress was tested through electrolyte leakage tests from the damaged leaves. SMI-11 was identified as the most cold-tolerant source followed by SMI-127 and L-17 with a slight increase in the electrolyte leakage compared to SMI-11. The identified inbreds offer a strong basis for developing cold-tolerant maize varieties and could be exploited as parental lines in hybrid development which may improve the resilience and productivity of maize under cold environments.

Keywords: *Maize, Inbreds, cold tolerancestress electrolyte leakage, CTR*

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops globally accounting for 40% (>800 mt) of the global food production (Yu T., 2022). It plays a vital role in food security, animal feed, and industrial applications. In India, maize is cultivated on an area of 10.04 with a production of 33.62 mt and a productivity of 3.49 t/ha. Maize in J&K is a rainfed crop (> 85% area) covering an area of 0.28 ha with a production of 0.56 mt and a productivity of 2 t/ha (Dar Z. A., 2024). Maize serves as a staple food for the vast population of Jammu and Kashmir, especially to tribal and nomadic people (DACNET, 2021). Despite its economic and nutritional significance, maize production is highly vulnerable to stresses in these mid-high altitude regions, such as cold stress(Ma Y. H., 2018). Climate change is a pressing global issue that poses significant challenges to agriculture and food security. Given the increasing frequency and intensity of cold spells in many maize-growing regions, it is imperative to develop maize varieties with enhanced cold tolerance.

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Maize is the most versatile and significant cereal crop grown across various agro-climatic zones within the state; however, it is highly sensitive to low temperatures(Enders T.A., 2019). Cold stress primarily hinders germination, seedling establishment, flowering, and grain filling, particularly in regions where early planting or high-altitude cultivation is essential (Li, 2019). Enhancing cold tolerance in maize can enable early sowing which would facilitate the crop to endure cold spells that occur in early spring. Early sowing would also result in an extended vegetative period, thus allowing the plants to accumulate additional biomass (Aydinoglu, 2020).

Crop's effectiveness is determined by the magnitude of genetic variability present in the population, but also by how heritable it is (Hussain *et al*., 2011). The exploration of genetic variability within maize inbred lines offers significant potential for breeding programs aimed at enhancing cold tolerance. Inbred lines, being genetically homozygous and stable, provide a robust platform for identifying traits and mechanisms linked to stress tolerance. Many potential traits have been proposed for screening for cold tolerance, such as ranking of plants based on growth rate or yield, and germination rate (Hotchkiss *et al*. 1997; Revilla *et al*. 2003),root morphology (Hund*et al*. 2008), leaf injury, and chlorophyll fluorescence (Fracheboud*et al*. 2004).

Out of these traits, screening based on physiological traits would be more important and relevant, as those techniques are more feasible in terms of reliability, speed, and cost-effectiveness. By analyzzing traits such as cold tolerance rating (CTR) and electrical conductivity test, researchers can uncover key indicators of cold stress resilience. Understanding trait associations is particularly important in breeding programs because it enables indirect selection for complex traits such as cold tolerance, which are influenced by multiple genes and environmental interactions.

Genetic variability among inbred lines acts as a repository of diverse traits, enabling the identification of genotypes well-suited for cold environments. Furthermore, understanding the interrelationships among traits plays a crucial role in accelerating the development of cultivars that integrate high-yield potential with enhanced stress tolerance (Kumar *et al.*, 2014).

Cold stress also results in membrane integrity leading to ice formation in plant tissues which leads to solute leakage. Electrolyte leakage is one of the earliest markers of cold damage and is widely used as a test for the stress-induced injury of plant tissues (Demidchik*et al.,* 2014), (Nayyar*et al.,* 2005)Notably, electrolyte leakage content increased significantly under cold stress conditions.

Keeping this in view, we characterized a set of 164 maize inbreds under cold stress conditions both in the field and under controlled conditions to screen for any damage symptoms caused by cold stress and used electrolyte leakage, an indicator to test cold injury in the stressed plant tissues (leaf). The comparison of different inbreds with the germplasm could be useful in identifying the relative potential of each inbred to cope with cold temperature stress.

Experimental site

The field experiments were conducted during the years 2022 and 2023 and laid at Mountain Crop Research Station, Larnoo, SKUAST-K, J&K, India (75.331°N, 33.644°E) at an altitude of 2280 msl. The study area location is characterized by cold temperate conditions with moderate summers and severe winters and at Greenhouse, Division of Genetics & Plant Breeding, FoA, Wadura(34°17 N,74°33 E) at an altitude of 1594 msl.

Plant Material

A set of diverse 164 maize inbred lines including 4 checks (Table 1) were used in the study. The seed material was procured from Mountain Crop Research Station (MCRS), Larnoo, Dryland Agriculture Research Station (DARS), Budgam, and the Indian Institute of Maize Research (IIMR), Ludhiana.

Table 1: List of inbreds used in the study

SMI-61	SMI-111	SMI-216	PW-1	SMI-24	SMI-178-1
CML-190	SMI-303	SMI-401	SMI-120	SMI-508	SMI-9
SMI-50	SMI-82	L-23	SMI-105	244-2	IMR-34
SMI-62	SMI-138	SMI-14-1	SMI-221	EC808943	MW-1
SMI-174-1	SMI131-1	SMI-6	IMR-387	SMI-64	SMI-114-1
SMI-31	EC-808941	SMI-15	SMI-27	SMI-11-2	SMI-92
SMI-83-1	L33	SMI-135-7	SMI-462	SMI-10	IMR-41
SMI-4	CML-128-1	SMI-219	CML-145	BP-1	EC-808955
SMI-45-5	SMI-56	SMI-157	L-17	SMI-39-2-8	EC-808965
SMI-24-2	SMI-130	SMI-162	W-3	LNK	EC-808949
SMI-339-1	SMI-153	SMI-219-1	L-13	SMI-59	C1 (Gurez)
SMI-39-2-7-1	SMI-133	PL-1	SMI-46-1	SMI-174	C2(EC808942-2)
SMI-360	KS-1	SMI-502	SMI-39-2-4-10	Y-4	C3 (Larnoo)
CML-145-1	SMI-53	SMI-67	SMI-112	SMI-39-3	C4 (EC-808944)
SMI-6-1	SMI-39-2-8-1	SMI-64-1	SMI-201	SMI-20	
SMI-48	IMR-12	SMI-98	EC-808944	SMI-24-1	
SMI-114-4	IMR-403	SMI-12	SMI-75	SMI-52	
SMI-30-1	IMR-414	KDM895A	SMI-125	SMI-13-S6	
CML-128	SMI-30	SMI-510	SMI-46	IMR-7	
TW-1	IMR-440	SMI-90	SMI-564-1	SMI-39-2	
SMI-222	SMI-18	SMI-39-2-7	SMI-39-2-4	SMI-123	
SMI-510-2	SMI-560	SMI-299-6	SMI-75-1	SMI-45	
SMI-201-1	SMI-339	SMI-25	SMI-161	IMR-8	
SMI-222-1	SMI-52	IMR-204	CML-141	SMI-61-5	
SMI-31	SMI-22	SMI-127	SMI-32	SMI-39	
SMI-11	SMI-102	SMI-26	PMI-105	CML-39-A	
IMR-207	TP-1	SMI-244	SMI-132	IMR-425	
SMI-43	SMI-14-2	SMI-131	SMI-5	SMI-460	
SMI-49	SMI-74	SMI-17	SMI-10-1	SMI-114-2	
SMI-109	EC-808943	SMI-13-S6-2	SMI-13	SMI-52-1	

Methodology

A set of diverse 164 maize inbred lines were evaluated for cold tolerance rating in the field under natural conditions in the month of April on a rating scale of 1 to 5 and the same methodology was followed for controlled conditions. It was scored on the basis of visible cold stress symptoms, i.e. inbreds with the least stress symptoms (chlorosis, necrosis/leaf firing, leaf etiolation, and stunted growth) were scored as 1 for cold tolerance and vice-versa (1-5 scale).

These inbreds were further assessed for chilling injury in the laboratory with an electrical conductivity test from the leaf tissues damaged by cold stress under controlled conditions.

Electrolyte leakage was measured using young leaf tissues of seedlings (Lutts*et al*., 1996). To remove any surface-adhered electrolytes, samples (1 gram) were first washed with deionized water. These were then placed in closed glass vials containing 10 ml of deionized water and were incubated at room temperature for thirty minutes with a constant shaking on a rotary shaker (150 rpm), followed by measuring the electrical conductivity of the solution (L1) in micro siemens/centimeter (μS/cm). Each sample was then autoclaved at 120°Celcius for 10 minutes and the electrical conductivity (L2) was again obtained after shaking for thirty minutes at room temperature. The electrolyte leakage index (ELI) was calculated as:

$$\boldsymbol{ELI}\ (\%) = (L1/L2) \times 100$$

The ELI represents the leakage of electrolytes from damaged plant tissues as the percent of the leakage from tissues completely destroyed after autoclave (100%).

Table 2: Cold Tolerance Rating (CTR) Scale

Scale	Symptoms	Category
1	No sign of damage	Highly Tolerant
2	Minimal damage, no discoloration, minimal wilting	Tolerant
3	Some discolouration, dry tissue	Moderately Tolerant
4	Intermediate level of leaf damage, discoloured and dry tissue, primarily on the edges and tips of the leaf	Susceptible
5	Severe leaf damage, large proportion of dry / dead tissue, severe wilting	Highly Susceptible

Results and Discussion

Cold stress poses a significant challenge to maize production in high-altitude regions such as Kashmir, where low temperatures hinder seed germination, seedling growth, and overall yield. With the increasing frequency and intensity of cold spells, assessing maize varieties for cold tolerance is essential. The development of cold-tolerant varieties can facilitate early sowing, allowing crops to withstand early spring cold spells and extend the vegetative period, thus enhancing biomass accumulation. Cell membrane permeability is changed by the disruption of cell membranes and this damage will cause the loss of electrolytes, ultimately resulting in the death of plant cells. Higher loss in electrolyte leakage implies less cell tolerance to temperature change and vice versa Wijewardana*et al*. (2016), Cold tolerance was assessed in 164 maize inbreds under the distinct climatic conditions of Kashmir using a rating scale of 1–5, complemented by electrolyte leakage (EL), a physiological index to quantify cellular damage following cold stress (Figure 1, 2 and 3). Significant variability in cold tolerance was observed among the inbreds. SMI-11 was identified as the most cold-tolerant genotype, with a cold tolerance rating (CTR) of 1 and an electrolyte leakage (EC%) of 22.11, followed by L-17 (CTR=1, EC% = 22.75) and SMI-127 (CTR=1, EC% = 28.57). In contrast, SMI-564-1 displayed moderate tolerance (CTR=2, EC% = 22.54), followed by Larnoo (CTR=2, EC% = 29.65), IMR-7 (CTR=2, EC% = 29.74), and SMI-48 (CTR=2, EC% = 29.75). Whereas, SMI-59 was identified as the most cold-susceptible genotype, with a cold tolerance rating (CTR) of 5 and an electrolyte leakage (EC%) of 85.45 followed by SMI-31 (CTR=5, EC% = 83.68) and SMI-39-2-8 (CTR=5, EC% =80.44). The electrolyte leakage analysis was in agreement with the observed data for cold tolerance rating suggesting more leakage of electrolytes in the identified cold susceptible accessions. Our results align with those of previous literature such as Wijewardana*et al*. (2016), who state that the cold-tolerant genotype exhibits significantly lower electrolyte leakage compared to cold-sensitive varieties.

Conclusion

A similar trend was observed between cold tolerance rating (CTR) and electrolyte leakage (EL), wherein inbreds exhibiting lower electrolyte leakage demonstrated superior performance in cold tolerance assessments. A significant variation was found between the inbreds for cold tolerance.

Among the 164 maize inbred lines assessed for cold tolerance, 7 genotypes were identified as Highly Tolerant, while 16 were categorized as Tolerant. The majority of the inbred lines, comprising 93 genotypes, were classified as Moderately Tolerant. Additionally, 42 genotypes were designated as Susceptible, and only 6 genotypes were classified as Highly Susceptible. SMI-11, L-17, SMI-127, SMI-564-1, Larnoo, IMR-7, and SMI-48 displayed superior cold tolerance and can be used in future breeding programs as parental lines in hybrid development which may improve the resilience and productivity of maize under cold environments. These inbreds warrant further investigation for their biochemical and physiological adaptations under cold stress. This can include transcriptomic and proteomic analyses to understand underlying mechanisms.

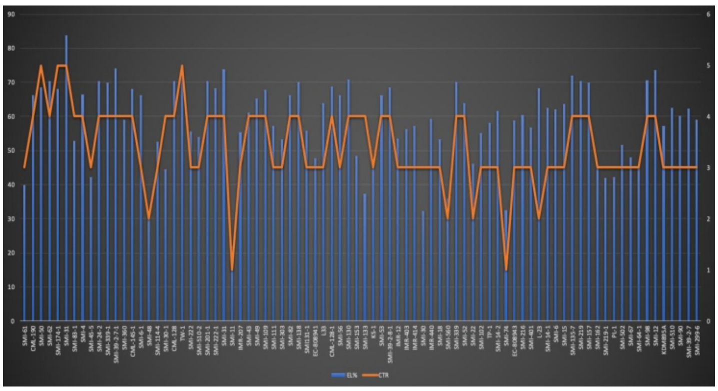

Figure 1: Performance Metrics Comparison: Electrolyte Leakage (EL%) vs Cold Tolerance Rating(CTR) Across Inbreds

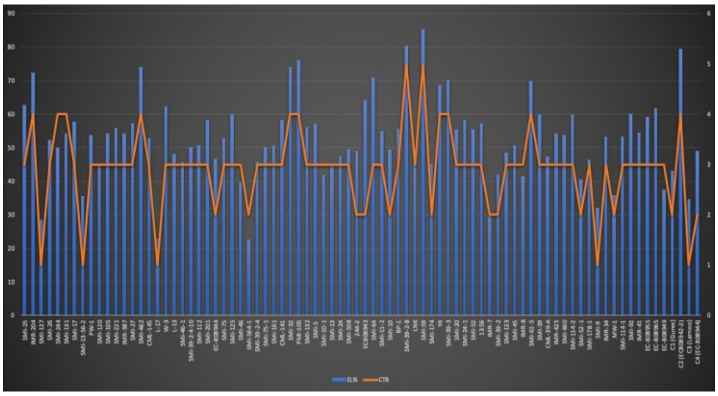

Figure 2: Performance Metrics Comparison: Electrolyte Leakage (EL%) vs Cold Tolerance Rating(CTR) Across Inbreds

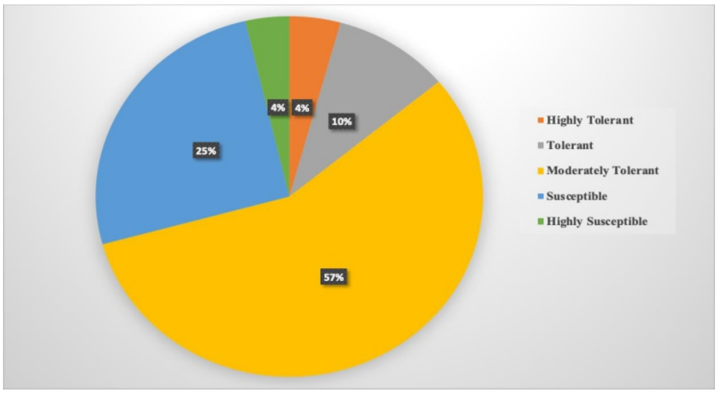

Figure 3: Distribution Of Maize Inbreds In Each Category

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