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# Winter Tolerance Potential of Genetically Diverse Sugarcane Clones under Subtropical Climate of Northern India

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**Abstract:** The low temperature (LT) conditions that prevail during winter in subtropical regions of India drastically affect the growth and yield of sugarcane. To identify low-temperature-tolerant agronomical acceptable genotypes for immediate deployment as donor parents in the subtropical sugarcane breeding program, 34 sugarcane clones belonging to 7 genetically diverse groups were evaluated under three crop environments, viz., spring planting, winter ratoon and spring ratoon, during 2015–2016 and 2016–2017. In the winter ratoon crop, commercial cane sugar and cane yield were reduced, whereas sucrose % was increased over the spring planted crop and the spring ratoon crop. The wild species and introgressed hybrid groups showed improvement for yield and quality traits in the winter ratoon crop, whereas commercial and near commercial groups showed reduction for these traits over the plant and spring ratoon crops. The tropical cultivars group was the poorest performer irrespective of the traits and crops. Yield per se under a stress environment was adjudged as the best selection criteria. For classification of sugarcane clones according to their low temperature tolerance, an index named winter tolerance index (WTI) is proposed which takes into account the winter sprouting index (WSI), winter growth and yield per se of the winter ratoon crop. The WTI had significant positive association with WSI, cane yield, millable cane population and cane length. As per the WTI ratings, the wild species of *Saccharum* complex and introgressed hybrid groups were rated as excellent WT clones. Subtropical commercial or advanced generation groups were poor WT clones, and tropical commercial cultivars group were winter sensitive clones. Clones such as AS04-635, AS04-1687, IK76-48, GU07-2276, IND00-1040, IND00-1038 and IND00-1039 had excellent tolerance, and GU07-3849, AS04-245, Co 0238, AS04-2097 and GU07-3774 had good WTI scores. The variety, Co 0238, may be continued for cultivation under LT regions with prophylactic measurers for red rot, while other clones listed above may be utilized in subtropical breeding programs.

**Keywords:** sugarcane; winter ratoon; spring ratoon; winter tolerance index; temperature stress



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## 1. Introduction

Sugarcane is an important cash crop, cultivated worldwide for industrial production of sugar and bio-ethanol [1]. Being a C4 crop, it thrives well in both tropical and subtropical regions of the world with a wide range of soils and temperature [2]. In a tropical climate, sugarcane passes through four distinct growth phases, viz., germination, tillering, grand growth and maturity, whereas under the subtropical climate of Northern India, the crop undergoes forced maturity at the onset of winter (during October to February) even if the crop was planted during summer (i.e., May planting after harvesting of wheat crop) [3]. The optimum temperature for sugarcane growth is about 35 °C, and temperatures below 20 °C significantly limit its growth. At temperature below 12 °C or 15 °C, virtually no growth occurs [4–6]. In the subtropical states of India, every year, the prevailing low-temperature (LT) stress (November to February) restricts the active cane growth period to 8–9 months from the normal period of 10–12 months (in tropical states of India), resulting in low cane yield and sugar recovery [7]. During LT stress, the sensitive cane varieties express several physio-chemical changes, viz., reduction of water/mineral uptake and photosynthetic rate, changes in membrane structure, disruption of ion homeostasis and accumulation of reactive oxygen species (ROS) [8]. LT stress significantly reduces the cane yield and prevents or delays bud sprouting and juice quality of sugarcane [9,10].

Temperature plays an essential role in synthesis and accumulation of sucrose in C4 plants such as sugarcane [11]. The production of phosphoenol pyruvate carboxykinase is sensitive to low temperatures in C4 plants [12]. In the course of low-temperature weather spells, juice acidity increases probably due to the formation of organic acids by the conversion of stored sucrose in the stalk [13]. In subtropical India during winter, below 10 °C temperature distresses the activities of sucrose-synthesizing and hydrolyzing enzymes, affecting the synthesis, movement and accumulation of sucrose, which results in a drop in sucrose recovery due to low-temperature-induced inversion [14]. The quality of sugarcane is principally articulated by the weather sequences met by the crop instead of its absolute age [11,15] at harvest; however, the chance of age with calendar months may be of some significance [16].

In the Northern India/subtropical region, autumn planted (October–November planting) and/or winter ratooned sugarcane crops (ratoon initiated during October–February) undergo chilling (<14 °C) and freezing stress (sub-zero) injury every year. In this region, the minimum temperature during winter dips to 2–3 °C, leading to very slow to no visible growth in terms of germination, tillering, sprouting of ratoon crop and elongation of clumps in most of the sugarcane varieties [17]. The farmers of subtropical states take only one ratoon crop due to prevailing low temperature conditions at the time of harvesting of the first ratoon crop during winter. Therefore, the ability to tolerate cold injury and the ability to sprout immediately after the harvest of the ratoon crop during winter would determine the success of sugarcane varieties in the subtropical region. Testing of cold or frost tolerance potential of promising sugarcane varieties and clones are being commonly performed in most of the countries that have low temperature conditions, viz., Australia, the United States of America [18,19], South Africa [20], Brazil [21,22], China [8,23,24] and India [17].

In order to develop sugarcane varieties with better winter ratooning ability, it is imperative to identify the potential donors with desirable traits to utilize in the sugarcane improvement programs. At ICAR-Sugarcane Breeding Institute, Coimbatore, India, over the years, several inter-specific and inter-generic hybrids have been developed, and their evaluation under subtropical conditions was thought as a worthwhile exercise in identifying valuable donors. Hence, the field study was planned to ascertain the degree of winter tolerance potential among diverse sugarcane clones.

## 2. Materials and Methods

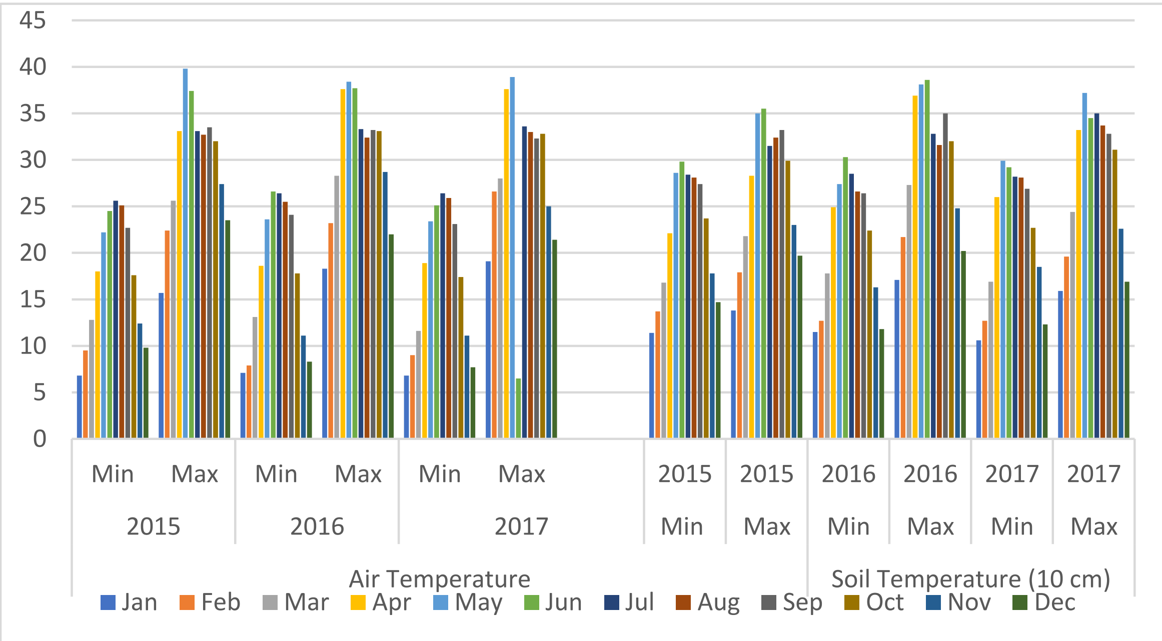
### 2.1. Experimental Plant Material and Layout

34 genetically diverse sugarcane clones including 8 subtropical early maturing cultivars (STEM), 7 subtropical mid-late maturing cultivars (STMM), 3 tropical commercial cultivars (TCC), 4 *S. spontaneum* introgressed hybrids (SSIH), 5 *Erianthus arundinaceous* cyto-nuclear introgressed advanced generation hybrids (EAIAGH), 3 inter-specific hybrids (ISH) derived from cold tolerant *S. spontaneum* collected from Arunachal Pradesh (GUC) and 4 *E. arundinaceus* and *S. spontaneum* basic species clones (EASS) were planted in 6 replications during the spring season (spring planted crop) in randomized complete block design during spring 2015–2016. Each clone was planted in two rows of six meter length at an inter row space of 0.9 m. Two budded sets @ 12 buds/m were used for planting. The recommended cultural practices for the region were followed to raise a good PC (plant crop). Half of the experimental area comprising three replications was harvested in the second fortnight of December 2015, while the remaining half was harvested during the first fortnight of March 2016, and ratoon crops (RC) were allowed to initiate winter-initiated ratoon crop (WIRC) and spring initiated ratoon crop (SIRC). The $ha^{-1}$ recommended dose

of major fertilizers, viz., N:P:K were applied @ 150N:50P:50K. The complete dose of P and K were applied as the based dose at the time of planting of the crop plant or management of the ratoon crop. A 1/4 dose of nitrogenous fertilizer was applied as a basal dose at the time of planting, whereas the remaining dose was top dressed in three splits at 40–45 days after planting or ratooning (DAP/R), 60–75 DAP/R and at the time of earthing up (100–120 DAP) in the plant crop and SIRC. In WIRC, the remaining 3/4 dose of nitrogenous fertilizer was top dressed in three splits at 75 days after rationing (DAR), 120–135 DAR and at the time of earthing up (150–160 DAR). The dose of nitrogenous fertilizer was kept 20% higher for the WIRC and SIRC.

### 2.2. Experimental Site, Topography and Climate

The field experiment was conducted at the Research Farm of ICAR Sugarcane Breeding Institute Regional Centre, Karnal, Haryana which is situated between 29.680 N latitude and 76.990 E longitude at 243 m above mean sea level. The soil of the site is well drained alluvial sandy loam, having mildly alkaline (7.5–7.8) pH. The weather parameters during the crop growth are given in Figure 1 (2015–2016 and 2016–2017).



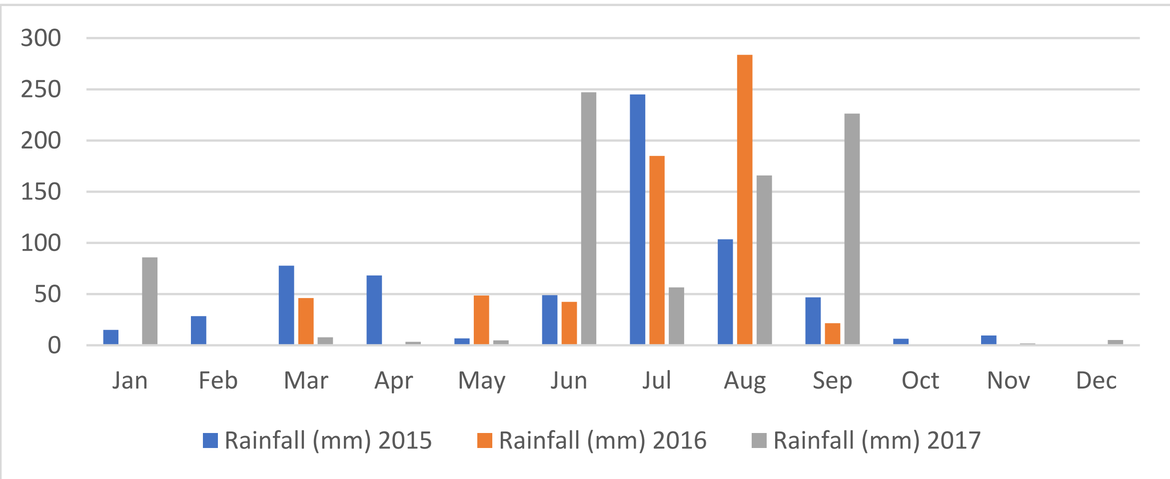




**Figure 1.** Weather data air and soil temp (°C) (min and max) and rainfall (mm) during 2015–2017.

### 2.3. Observations Recorded

In the PC trials, observations on metric traits, viz., germination percentage at 45 days after planting (DAP), number of tillers per plot at 120 DAP, number of millable canes per plot (NMC) at 240 DAP, stalk length, stalk diameter, single cane weight (SCW) and cane yield at 10th month were recorded. Juice analysis was carried out using five randomly selected stalks at the 10th month after planting; brix%, pol% and purity% of juice were estimated in the laboratory as per ICUMSA methods [25].

### 2.4. Winter Tolerance Index (WTI)

To study the winter sprouting (WS) potential of the clones, in three replications stalks of plant crops were cut at ground level during last week of December 2015 (coinciding with peak winter). This block of three replications was reserved for taking observations in the winter-initiated ratoon crop (WIRC), whereas the remaining three replications were harvested during the first fortnight of March to take observations in the spring-initiated ratoon crop (SIRC). The December ratooned stubbles were allowed to sprout during winter (December to February). The number of stubbles (clumps) alive and sprouted per plot and the number of shoots formed in each clump during winter were counted 45 days after ratooning, coinciding with the onset of spring (February). The winter sprouting index (WSI) was worked out following the formula suggested by Ram et al. [17] as follows:

$$\text{Winter Sprouting Index} = \frac{\%\text{ alive and sprouted clumps plot}^{-1} \times \text{ No of sprouts clump}^{-1}}{100}$$

To observe the active growth during winter, the stalks of unharvested replications were marked at top visible dewlap during mid-November, and the winter elongation growth (cm) was measured during the first week of March at the time of harvesting of the crop for SIRC.

The WIRC and SIRC were raised during the 2016–2017 crop season by following normal package and practice of cane cultivation of the region. Observations on metric traits, viz., NMC per plot, stalk length, stalk diameter, SCW and cane yield were recorded during November 2016. Juice analysis was carried out using five randomly selected stalks in SIRC and WIRC trials at the 8th and the 10th month after ratooning (coinciding with November) to record brix%, pol% and purity% in juice.

Based on cane yield of WIRC, WSI and plant elongation in winter months, an index named the Winter Tolerance Index (WTI) is proposed as shown below:

$$\text{Winter tolerance index} = \frac{\text{WSI } \times \text{ plant elongation during winter } \times \text{ Cane Yield t ha}^{-1}}{1000}$$

On the basis of WTI, sugarcane genotypes were grouped into five categories as shown below.

- WTI values: Tolerance category;
- >3.0: Excellent winter tolerance;
- 2.01–2.99: Good winter tolerance;
- 1.51–1.99: Average winter tolerance;
- 0.51–1.49: Poor winter tolerance;
- <0.50: Low temperature sensitive.

### 2.5. Statistical Analysis

The metric data were statistically analyzed using the SAS software version 9.3 (Strengthening Statistical Computing for NARS (iasri.res.in), and the means of different groups and genotypes were compared by critical difference (C.D.) test at 5% level of significance.

## 3. Results

The mean sum of square (MSS) for crop nature (PC and WIRC; WIRC crop and SIRC), genotypes and interaction (genotypes $\times$ ratooning season) were significant for all the studied traits.

### 3.1. Comparison of Plant and Ratoon Crop Mean

The overall mean for CCS yield was significantly lower in WIRC (6.93 t ha$^{-1}$) compared to SIRC (7.83 t ha$^{-1}$), and it was non-significant between PC (7.88 t ha$^{-1}$) and SIRC. Lower cane yield was recorded in WIRC (73.10 t ha$^{-1}$) compared to PC (77.9 t ha$^{-1}$), whereas the cane yield of SIRC (81.7 t ha$^{-1}$) was on par with that of PC. In addition to the differences in cane and sugar yield between PC and WIRC, PC recorded higher millable canes, single cane weight and cane length than ratoon crops (WIRC and SIRC). Although the cane yield of WIRC was lower than the PC, the WIRC recorded significantly higher pol% (14.3%) than plant crop (14.1%) (Table 1).

### 3.2. Comparison of WIRC and SIRC Mean

Sugar yield, cane yield and NMC recorded 11.49%, 10.53% and 6.58% improvement, respectively, in SIRC over WIRC, whereas Pol% was 2.14% higher in WIRC (Table 1). The higher pol% in juice in WIRC than SIRC might be due to an increase in crop age under WIRC. The LT prevailed during the sprouting time of WIRC adversely affected the NMC (an important yield contributing trait). As a result, the value of dependent traits, viz., cane and CCS yield were reduced.

### 3.3. Inter Comparison of the Groups

The commercial clones, viz., subtropical early maturing cultivars (STEM) and subtropical mid-late maturing cultivars (STMM), produced significantly higher CCS yield (t ha$^{-1}$), pol% and SCW in PC than WIRC and SIRC over their respective experimental means (Table 1). Similarly, these genetic groups (except STEM in WIRC) produced significantly higher cane yield over the respective experimental means of the crop environments (PC, WIRC and SIRC). However, tropical commercial cultivars (TCC) did not perform well under the subtropical environment as indicated by their lower mean values for all the traits under all crop types studied. *E. arundinaceus* cyto-nuclear genome introgressed advanced generation hybrids (EAIAGH) were another important group which produced significantly higher CCS yield (10.27 t ha$^{-1}$), cane yield (95.00 t ha$^{-1}$), pol (14.30% in PC and 14.80% in WIRC), SCW (in PC 0.99 kg; SIRC 0.88 kg; WIRC 0.89 kg) and cane length (242.80 cm, 231.80 cm and 266.30 cm in PC, WIRC and SIRC, respectively) over the experimental mean, but significantly lower for pol% than STEM and STMM groups. It is to be noted that most of the clones belonging to the EAIAGH group did not perform well in WIRC and SIRC for CCS and cane yield.

The number of millable canes was lower in the commercial (STEM, STMM, TCC) and the near commercial (EAIAGH) group when compared to their respective experimental means of test environments (PC, WIRC and SIRC). Three groups, viz., SSIH (*S. spontaneum* introgressed immediate hybrids), EAIAGH and GUC (germplasm utilized clones) reported significantly higher cane length (cm) over the experimental mean and all other groups in all the three crop types. The cane yield of the SSIH and GUC group was significantly higher in WIRC over the experimental mean, while the former group was on par (PC and SIRC), and the latter group had either lower (PC) or higher (SIRC) performance over respective means of the studied environment. The EASS (*E. arundinaceus* and *S. spontaneum* species clones) group was the poor performer for CCS yield, cane yield, pol% and SCW in all the crop type studied (PC, WIRC and SIRC) but was the best performer for NMC (Table 1).

**Table 1.** Performance of different categories of sugarcane clones for cane yield and juice quality traits in plant and ratoon crops.

| Genetic Group | Traits | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CCS (t ha$^{-1}$) | | | Cane Yield (t ha$^{-1}$) | | | NMC ('000/ha) | | | Pol% 10 M | | | SCW (kg) | | | Cane Length (cm) | | |
| | PC | WIRC | SIRC | PC | WIRC | SIRC | PC | WIRC | SIRC | PC | WIRC | SIRC | PC | WIRC | SIRC | PC | WIRC | SIRC |
| STEM | 11.76 * | 10.05 * | 10.91 * | 92.8 * | 75.9 | 86.6 * | 89.2 | 76.8 | 83.5 | 18.1 * | 18.8 * | 18.5 * | 1.04 * | 0.99 * | 1.04 * | 231.0 | 223.7 | 218.5 |
| STMM | 9.7 * | 9.3 * | 10.45 * | 81.2 | 79.8 * | 91.4 * | 83.0 | 80.7 | 85.7 | 17.2 * | 17.0 * | 16.5 * | 0.98 * | 0.99 * | 1.07 * | 206.9 | 195.7 | 196.2 |
| TCC | 7.44 | 3.49 | 5.84 | 66.5 | 30.1 | 52.5 | 75.7 | 47.1 | 69.4 | 15.9 * | 16.6 * | 16.2 * | 0.88 * | 0.64 | 0.76 * | 209.4 | 185.8 | 178.3 |
| *SSIH* | 4.27 | 4.85 | 4.28 | 79.5 | 93.7 * | 80.5 | 125.8 * | 146.6 * | 131.4 * | 8.7 | 8.7 | 8.8 | 0.63 | 0.64 | 0.61 | 285.5 * | 271.7 * | 261.2 * |
| *EAIAGH* | 10.27 * | 6.94 | 7.64 | 95.0 * | 66.5 | 76.1 | 95.5 | 75.4 | 85.8 | 14.3 * | 13.9 | 14.8 * | 0.99 * | 0.88 * | 0.89 * | 242.8 * | 231.8 * | 226.3 * |
| GUC | 3.49 | 5.03 | 7.81 | 67.8 | 89.1 * | 91.8 * | 126.3 * | 163.6 * | 208.2 * | 9.4 | 11.1 | 10.1 | 0.54 | 0.54 | 0.44 | 238.9 * | 237.2 * | 233.3 * |
| *EASS* | 2.18 | 3.55 | 2.37 | 35.3 | 63.3 | 60.5 | 140.2 * | 283.0 * | 281.9 * | 8.1 | 7.5 | 6.8 | 0.25 | 0.22 | 0.21 | 193.8 | 207.9 | 198.7 |
| **Mean** | **7.88** | **6.93** | **7.83** | **77.9** | **73.1** | **81.7** | **101.2** | **114.9** | **123.0** | **14.1** | **14.3** | **14.0** | **0.76** | **0.70** | **0.72** | **229.7** | **222.0** | **216.1** |
| **CD 5%** | | | | | | | | | | | | | | | | | | |
| Season | | 0.54 | | | 6.4 | | | 4.29 | | | 0.14 | | | 0.015 | | | 4.29 | |
| Group | | 0.4 | | | 4.66 | | | 5.56 | | | 0.2 | | | 0.022 | | | 6.56 | |
| Interaction | | 0.69 | | | 8.08 | | | 11.36 | | | 0.4 | | | 0.039 | | | 11.36 | |

Note: CCS = commercial cane sugar; NMC = number of millable cane; SCW = single cane weight; PC = plant crop; WIRC = winter-initiated ratoon crop; SIRC = spring initiated ratoon crop; STEM = subtropical early maturing clones; STMM = subtropical mid late maturing clones; TCC = tropical commercial clones; SSIH = *Saccharum sponteneum* introgressed hybrids; EAIAGH = *Erianthus arundinaceus* cyto-nuclear introgressed advanced generation hybrids; GUC = germplasm utilized clones; EASS = *Erianthus arundinaceus S. spontaneum.* * indicates significantly superior over experimental mean.

### *3.4. Inter-Crop Environmental Comparison of the Groups*

#### 3.4.1. Subtropical Early Maturing Commercial Cultivars (STEM)

Commercial type early varieties recorded significantly higher sugar and cane yield and NMC population in the plant crop than the ratoon crops, and between ratoons, it was higher in SIRC than WIRC (Table 1). The performance trend for pol% was the reverse, where WIRC (18.80%) had higher performance over SIRC (18.5%) and PC (18.10%). The SCW was on par between PC (1.04 kg) and SIRC (1.04 kg), and their performance was higher over WIRC (0.99 kg). The cane length was significantly higher in PC (231.00 cm) and WIRC (223.70 cm) and had on par performance with SIRC (218.50 cm).

#### 3.4.2. Subtropical Mid-late Maturing Commercial Cultivars (STMM)

The group had clear-cut differences for the expression of CCS t ha$^{-1}$ in different crop environments. The highest CCS yield was exhibited by SIRC (10.45 t ha$^{-1}$), followed by PC (9.70 t ha$^{-1}$) and WIRC (9.30 t ha$^{-1}$). The cane yield of the group was the highest in SIRC (91.40 t ha$^{-1}$) over PC (81.20 t ha$^{-1}$) and WIRC (79.80 t ha$^{-1}$). The latter two growth environments had insignificant differences. The pol% in descending order was PC (17.20%), followed by WIRC (17.00%) and SIRC (16.50%). The heaviest SCW was attained by the group in SIRC (1.07 kg), followed by WIRC (0.99 kg) and PC (0.98 kg). The trend for cane length in descending order was PC (206.9 cm), followed by WIRC (195.6 cm) and SIRC (196.2 cm).

#### 3.4.3. Tropical Commercial Cultivars (TCC)

The group had clear differences for the important traits in all three crops. The performance for cane yield and its contributing traits was very high in PC over SIRC and WIRC. The WIRC had extremely low performance for CCS yield (113.2% less over PC; 67.3% less over SIRC), cane yield (120.9% less over PC; 74.4% less over SIRC), NMC (60.7% less over PC; 47.35% less over SIRC) and SCW (37.5% less over PC; 18.75% less over SIRC).

#### 3.4.4. *S. spontaneum* Introgressed Immediate Hybrids (SSIH)

The group had better performance under WIRC for CCS (t/ha), cane yield and NMC population over PC, with an improvement of 13.58%, 17.86% and 16.53%, respectively. There were no significant differences among the crop environments for SCW and pol%. Similarly, the differences between PC and SIRC were insignificant for most of the studied traits. This indicates that the group has better winter tolerance potential.

#### 3.4.5. *E. arundinaceus* Cyto-Nulcear Genome Introgressed Advanced Generation Hybrids (EAIAGH)

The best performance of the group for CCS yield (10.27 t ha$^{-1}$), cane yield (95.00 t ha$^{-1}$), NMC (95.5′000/ha), SCW (0.99 kg) and cane length (242.80 cm) was observed in PC, whereas the worst performance was recorded in WIRC with a reduction of 47.98%, 42.86%, 16.53% and 4.70% for CCS yield, cane yield, NMC and cane length, respectively. The pol% values were significantly higher in SIRC (14.80%) compared to PC (14.30%) and WIRC (13.90%).

#### 3.4.6. Germplasm Utilized Clones (GUC)

The performance for CCS yield, cane yield and NMC, in descending order, was in SIRC (CCS yield: 7.81 t ha$^{-1}$; cane yield: 91.80 t ha$^{-1}$ and NMC: 208.20 thousands/ha) followed by WIRC (CCS yield: 5.03 t ha$^{-1}$; cane yield: 89.1 t ha$^{-1}$ and NMC 163.6 thousands/ha) and PC (3.49 t ha$^{-1}$ CCS yield; 67.8 t ha$^{-1}$ cane yield and 126.3 thousands/ha NMC). Pol% was the highest in WIRC (11.1) followed by SIRC (10.1) and PC (9.4). The better performance of ratoon crops (WIRC and SIRC) over PC for cane yield and juice quality traits.

#### 3.4.7. *E. arundinaceus* and *S. spontaneum* Species Clones (EASS)

The WIRC produced significantly higher CCS yield (3.55 t $ha^{-1}$), cane yield (63.30 t $ha^{-1}$), NMC population (283.00 thousands/ha) and cane length (207.90 cm) over PC. The highest improvement was observed in the NMC population in WIRC (101.85%) and SIRC (101.07%) compared to PC.

### *3.5. Identification of Traits for Winter Tolerance*

The winter tolerance index had strong and positive association with WSI (0.62 **), cane yield (0.49 **), NMC (0.57 **) and cane length (0.46 **), whereas its association with CCS yield was insignificant (Table 2). On the contrary, single cane weight (−0.35 *), cane diameter (−0.45 **) and pol% −0.57 **) had negative association with WTI. The association of WSI was significant and positive with NMC (0.80 **), whereas it was insignificant with CCS yield, cane yield and cane length. WSI showed negative association with SCW (−0.63 **), cane diameter (−0.71 ** and pol% (−0.76 **).

**Table 2.** Pearson Correlation coefficients between Winter tolerance index, Winter sprouting index, yield and quality parameters.

| | **WTI** | **WSI** | **CCS t $ha^{-1}$** | **Cane Yield** | **NMC** | **SCW** | **Cane Dia** | **Cane Length** | **pol%** |
|---|---|---|---|---|---|---|---|---|---|
| WTI | 1 | | | | | | | | |
| WSI | 0.62 ** | 1 | | | | | | | |
| CCS t $ha^{-1}$ | −0.10 NS | −0.30 NS | 1 | | | | | | |
| Cane yield | 0.49 ** | 0.20 NS | 0.65 ** | 1 | | | | | |
| NMC | 0.57 ** | 0.80 ** | −0.28 NS | 0.09 NS | 1 | | | | |
| SCW | −0.35 * | −0.63 ** | 0.65 ** | 0.37 * | −0.76 ** | 1 | | | |
| Cane Dia | −0.45 ** | −0.71 ** | 0.55 ** | 0.17 NS | −0.84 ** | 0.91 ** | 1 | | |
| Cane length | 0.46 ** | 0.22 NS | 0.19 NS | 0.70 ** | 0.03 NS | 0.27 NS | 0.09 NS | 1 | |
| pol% | −0.57 ** | −0.76 ** | 0.52 ** | −0.12 NS | −0.69 ** | 0.62 ** | 0.76 ** | −0.37 * | 1 |

Note: WTI = winter tolerance index; WSI = winter sprouting index; * and ** refers to significant at $p$ = 0.01 and 0.001.

The correlation of other variables indicates that the cane yield had strong and positive association with CCS yield (0.65 **) and cane length (0.69 **), whereas its association with NMC, cane diameter and pol% were non-significant. Pol% had positive association with CCS t $ha^{-1}$ (0.52 **), SCW (0.62 **) and cane diameter (0.75 **), whereas with NMC (−0.69 **) and cane length (−0.36 *), it had negative association.

### *3.6. Classification of Groups Based on Winter Tolerance and Winter Sprouting Index*

The WTI and WSI scores of SSIH, EASS and GUC groups indicate the excellent winter tolerance and winter sprouting potential of these groups (Table 3). When comparing both the indexes, WTI indicates the superiority of SSIH (5.66) group over EASS (3.71) and GUC (3.00), whereas per WSI, the group EASS (5.05) was the best performer followed by SSIH (4.09) and GUC (3.96). The WTI indicated the superiority of the EASS group over GUC, but WSI weighted both these group as on par performers.

Groups such as STEM, STMM and EAIAGH had insignificant differences among themselves and were classified as poor winter tolerant as well as poor winter sprouting categories. The TCC was the poorest performer for both the indexes and was classified as a winter sensitive category. Their WTI values were low due to poor WIRC yield, lesser elongation of plant during winter month and poor WSI.

**Table 3.** Classification of sugarcane genotypic groups on the basis of WTI and WSI.

| Genetic Group | WTI Scores | Interpretation | WSI Scores | Interpretation | % Sprouted | Sprouts Clump$^{-1}$ | Winter Elongation |
|---|---|---|---|---|---|---|---|
| STEM | 0.94 | Poor (0.50–1.49) | 1.62 | Poor (1.01–1.99) | 83.96 | 1.87 | 6.6 |
| STMM | 0.9 | Poor (0.50–1.49) | 1.63 | Poor (1.01–1.99) | 76.41 | 2.07 | 5.4 |
| TCC | 0.06 | Low temp sensitive (<1.0) | 0.54 | Low temp sensitive (<1.0) | 60.04 | 0.87 | 4.0 |
| SSIH | 5.66 | Excellent winter tolerance (>3.0) | 4.09 | Excellent (>3.0) | 91.4 | 4.42 | 13.2 |
| EAIAGH | 0.69 | Poor (0.50–1.49) | 1.54 | Poor (1.01–1.99) | 69.94 | 2.13 | 6.6 |
| GUC | 3.0 | Excellent winter tolerance (>3.0) | 3.96 | Excellent (>3.0) | 94.96 | 4.16 | 9.4 |
| EASS | 3.71 | Excellent winter tolerance (>3.0) | 5.05 | Excellent (>3.0) | 93.41 | 5.4 | 12.7 |
| GM | 2.14 | | 2.63 | | 81.44 | 2.99 | 8.27 |
| CD | 0.39 | | 0.30 | | 2.48 | 0.28 | 0.67 |
| CV | 10.79 | | 6.33 | | 1.69 | 5.29 | 4.48 |

### *3.7. Identification of Winter Tolerant Clones*

As per the WTI scores, seven clones, viz., AS04-635 (12.51), AS04-1687 (5.27), IK76-48 (4.39), Gu07-2276 (4.13), IND00-1040 (3.73), IND00-1038 (3.49) and IND00-1039 (3.24) were identified excellent for their winter tolerance potential, while five clones, viz., Gu07-3849 (2.96), AS04-245 (2.92) and Co 0238 (2.54), AS04-2097 (2.13) and Gu07-3774 (2.13) were identified good for their winter tolerance potential (Table 4). Two subtropical cultivars, viz., Co 98014 and Co 1148 were categorized as average winter tolerant ones.

**Table 4.** Classification of sugarcane genotypes into different winter tolerance categories based on WTI scores.

| Category of WTI | No of Genotypes | Genotype Names |
|---|---|---|
| Excellent winter tolerance (>3.0) | 7 | AS 04-635 (12.51), AS 04-1687 (5.27), IK 76-48 (4.39), Gu 07-2276 (4.13), IND00-1040 (3.73), IND00-1038 (3.49), IND00-1039 (3.24) |
| Good winter tolerance (2.0–2.99) | 5 | Gu 07-3849 (2.96), AS 04-245 (2.92), Co 0238 (2.54), AS04-2097 (2.13), Gu 07-3774 (2.13) |
| Average winter tolerance (1.50–1.99) | 2 | Co 98014 (1.84), Co 1148 (1.58) |
| Poor (0.50–1.49) | 10 | Co 05011 (1.38), CYMA09-1268 (1.34), Co 05009 (0.95), Co 06034 (0.89), Co 7717 (0.86), CYM07-986 (0.82), Co 89029 (0.68), CYMA09-1447 (0.62), CoS 767 (0.53), Co 89003 (0.53) |
| Low temperature sensitive (<0.50) | 10 | CoJ 64 (0.43), CYMA10-948 (0.39), Co 0118 (0.37), CYMA10-1460 (0.28), Co 0124 (0.17), CoS 8436 (0.16), Co 0237 (0.15), Co 6811 (0.08), Co 453 (0.06), Co 419 (0.03) |

All the excellent and good performer clones except Co 0238 are from wild or semi wild genotypic groups, viz., SSIH (AS04-635, AS04-1687, AS04-245, AS04-2097), EASS (IK76-48, IND00-1040, IND00-1038, IND00-1039) and GUC (Gu07-2276, Gu07-3849, Gu07-3774). In addition to these, commercial varieties Co 98014 and Co 1148 scored average WTI values.

## 4. Discussion

The significance of MSS for PC and WIRC; WIRC and SIRC for most of the yield and quality traits, viz., CCS (t/ha), cane yield (t/ha), NMC (′000/ha), SCW (kg), cane diameter (cm), brix%, pol%, purity% and CCS% indicated that the season of ratooning caused differential phenotypic expression due to temperature and year effects. While comparing the PC and ratoon crops, the CCS yield was significantly lower in WIRC (6.93 t $ha^{-1}$) compared to SIRC (7.83 t $ha^{-1}$). A similar trend was noted for cane yield, NMC, single cane weight and cane length, indicating the ill effect of LT in the winter-initiated ratoon crop. The varying response of sugarcane genotypes for sprouting, NMC and CCS in the ratoon crop was also reported by earlier researchers [26,27]. In contrast to yield and contributing traits, the WIRC recorded significantly higher pol% (14.3%) than the plant crop (14.1%). This finding is in conformity with that of Yadava [28] who reported better juice quality vis-à-vis sugar recovery in the ratoon crop over the plant crop.

The season of ratooning had a clear impact on the expression of important cane yield and juice quality traits. Sugar yield, cane yield and NMC recorded significant improvement in SIRC over WIRC, but pol% was higher in WIRC. The higher pol% in juice in the WIRC than the SIRC is due to an increase in crop age under WIRC. Ram et al. [16], while studying the pattern of sugar accumulation and yield performance in autumn and spring planted sugarcane varieties, also observed that the extended period yielded higher sugar content in the autumn crop over the spring planted crop. The LT prevailing during the sprouting time of WIRC adversely affected the NMC (an important yield contributing trait). As a result, the value of dependent traits, viz., cane and CCS yield, were reduced. Hasan et al. [29] also observed that SIRC was found to be superior over WIRC in the expression of most traits and genetic potential of genotypes.

### *4.1. Comparison between Different Groups for Cane Yield and Juice Quality*

The commercial type groups STEM and STMM produced significantly higher CCS yield (t $ha^{-1}$), pol% and SCW in PC over RC. Similarly, these genetic groups (except STEM in WIRC) produced significantly higher cane yield across the crop environments (PC, WIRC and SIRC). The better performance of these groups for sugar and cane yield is due to their selection history because in the past they were selected for these traits at the studied location. Phenotypic selection in the target environment might have captured the favorable alleles in these group of genotypes for important economic traits [30]. However, tropical commercial cultivars (TCC) did not perform well under a subtropical environment as indicated by their lower mean values for all the traits under all crop types studied. The poor performance of tropical commercial varieties is due to the fact that they were selected under a tropical environment, where LT stress does not prevail [31]. EAIAGH was another important group that performed better over general mean for the important traits. It is to be noted that most of the clones belonging to the EAIAGH group did not perform well in WIRC and SIRC for CCS and cane yield. In our earlier study dealing with the *E. arundinaceus*, *S. spontaneum* cyto-nuclear genome introgressed hybrids, a clear-cut difference in the performance for cane yield and juice quality traits was observed among different groups and backcross generations [31,32]. The differential response of the genotypes for ratooning ability was also reported earlier by Bhatnagar et al. [26] and Rafiq et al. [33]; hence ratooning ability of sugarcane cultivar is a function of genotype and environment interaction. Therefore, for incorporating the LT tolerance such a kind of advanced generation segregating material should be evaluated under endemic LT conditions of a subtropical climate.

The NMC population in STEM, STMM, TCC and EAIAGH was lower over the general mean (GM) and SSIH, GUC and EASS groups, irrespective of crop environments (PC, WIRC and SIRC). This is due to the fact that the commercial type genotypes due to their skewed selection towards increased cane thickness and the negative linkage between cane diameter and NMC (−0.84 correlation in our study) have reduced the NMC population [34,35] over their wild and semi-wild companion groups such as EASS, SSIH and GUC, which produced extremely higher NMC. In a move towards broadening the genetic base of Indian working germplasm, many wild genetic resources of *Saccharum* spp., such as *S. officinarum*, *S. robustum*, *S. spontaneum* as, *E. arundinaceus* and *Erianthusbengalense*, were utilized [30,31,36,37]. These and other similar earlier efforts have continually enriched the genetic diversity in the sugarcane cultivars. The wild genetic material and introgressed hybrids had below average performance for juice quality and cane yield traits, indicating that they need to be further crossed/backcrossed with commercial hybrids. Further, the better performance of GUC, EASS for NMC indicates that the wild germplasm and introgressed hybrids had a higher rate of genetic gain for the NMC over cane thickness and SCW [17,38,39].

### 4.2. Identification of Traits for Winter Tolerance

Yield per se is the ultimate selection criteria of any crop improvement program, but yield itself is a dependent trait controlled by several interdependent traits and is highly influenced by environmental conditions. To identify low-temperature-tolerant sugarcane genotypes under endemic subtropical conditions, an index called the winter sprouting index is available [17]. However, this index gives emphasis only to sprouting potential of the genotypes, whereas cane length, cane diameter and cane weight, etc., are the other contributing traits to cane yield. Therefore, in the present study, while computing WTI, cane yield, winter sprouts and winter growth are integrated together, WTI had strong positive association with WSI (0.62 **), cane yield (0.49 **), NMC (0.57 **) and cane length (0.46 **), whereas its association with CCS yield was insignificant (Table 2). Contrary to this SCW (−0.35 *), cane diameter (−0.45 **) and pol% (−0.57 **) had negative association with WTI, indicating that the WSI, cane yield, NMC and cane length were the positively influencing traits for winter tolerant potential in sugarcane. The strong and positive association of WTI with WSI, cane yield, NMC and cane length indicates that WTI can be an effective index in identifying better cane-yielding winter tolerant genotypes. It is pertinent to explain that the WSI had positive association with NMC (0.8 **) but did not exhibit significant association with the CCS yield and cane yield. Non-significant (in PC) and weak association (in RC) of WSI with CCS yield and cane yield was observed by Ram et al. [17]. The weak association indicates that better winter sprouting potential does not guaranteed better sugar and cane yield. However, the significant association of WTI with cane yield indicates that it can serve as a better index in the selection of high yielding winter tolerant genotypes. The correlation among the other variables confirm the findings of earlier researchers [34,35,38,39].

### 4.3. Classification of Groups and Clones Based on WTI and Mechanism of WT

To delineate the genotypes into tolerance or sensitive classes, various tolerance indices are under use, viz., Yield stability index [40], Yield index [41], Stress tolerance index [42], Geometric mean productivity [42], Stress susceptibility index [43], Mean productivity [44], Tolerance index [44], etc. All these indices are derived from yield only, but in reality, numerous contributing dependents/independents traits with varying magnitudes are responsible for the final expression of the yield [45]. To classify the sugarcane genotypes into true tolerance and sensitive classes against salinity, earlier we developed an index, viz., the Salinity Tolerance Index (STI) by adding up the percent reduction in the expression of six differentially expressed traits, viz., cane yield, NMC, SCW, cane height, cane diameter, juice extraction and tiller population under the highest stress level [45]. During LT stress, sugarcane almost stops visible growth, but if the underground soil temp (up to 10 cm), is above 12–15 °C, the rhizosphere shows growth in terms of producing sprouts. The winter sprouting index (WSI) proposed by Ram et al. [17] considers the sprouted clumps and no. of sprouts per clump only, but in reality, many genotypes continue to elongate. Further, cane yield, the ultimate trait of economic importance, is not taken into account in WSI, so we proposed the winter tolerance index (WTI) as mentioned in the Materials and Methods section. While classifying various categories of WTI, we considered that an ideal tolerant genotype should have >85 percent sprouted clumps, on average 4.5 sprouts per clump, nearly 10 cm plant elongation and should produce above average cane yield, i.e., >80 t $ha^{-1}$. In such case, the WTI, will be >3.0. So, the genotypes or group of genotypes having >3.0 WTI were categorized as excellent WT. The genotype or group of genotypes that have >80% sprouted clumps, an average of 4 sprouts per clump, 8 cm plant elongation and produce cane yield nearly 80 t $ha^{-1}$, has a WTI $\geq$2.0; we classified these into the good winter tolerance category. In a similar fashion, the rest of the categories of WTI were derived.

The WTI scores of SSIH, EASS and GUC groups indicate the excellent winter tolerance potential of these groups (Table 3). Groups such as STEM, STMM and EAIAGH had insignificant differences among themselves and were classified as poor winter tolerant. The TCC was the poorest performer and was classified as winter sensitive because of poor WIRC yield, lesser elongation of the plant during winter and poor WSI. These clones were not selected for a subtropical climate; hence it was not surprising to observe poor sprouting during winter in them [17].

As per WTI, seven genotypes, viz., AS04-635 (12.51), AS04-1687 (5.27), IK76-48 (4.39), Gu07-2276 (4.13), IND00-1040 (3.73), IND00-1038 (3.49) and IND00-1039 (3.24) were classified as excellent, while five genotypes, viz., Gu07-3849 (2.96), AS04-245 (2.92) and Co 0238 (2.54), AS04-2097 (2.13) and Gu07-3774 (2.13) were identified as good (Table 4). All the excellent and good performer clones except Co 0238 are from wild or semi-wild genotypic groups, viz., SSIH (AS04-635, AS04-1687, AS04-245, AS04-2097), EASS (IK76-48, IND00-1040, IND00-1038, IND00-1039) and GUC (Gu07-2276, Gu07-3849, Gu07-3774). Because of good winter tolerance potential, Co 0238 at present occupies nearly 2.77 m ha (highest area by any sugarcane variety of the world) of area in subtropical states having LT conditions during winter [46]. The sugarcane clones with wild relatives, viz., *Erianthus* or *S. spontaneum* as one of their immediate parents had excellent WSI (>3.0) scores [17]. Brandes [47] and Irvine [48] suggested utilization of wild species *Saccharurm spontaneum* and related genera such as *Erianthus bengalensis* in breeding for cold tolerance. Ram and Sahi [39] recorded 98.61% sprouting during winter in *S. spontaneum* clones, 84.17% in *S. barberi* clones and poor sprouting (19.24%) in *S. robustum* clones in a subtropical region of India. Glowacka et al. [49] reported cold tolerance in *S. spontaneum* × *Miscanthus* hybrid (Miscane-US84-1058). Hassan et al. [29] in a study on the ratooning potential of sugarcane genotypes under varying harvesting times (November, December, January, February, March) of a plant crop observed varying response among the genotypes for number of sprouts, millable cane, striped cane yield and sugar yield.

Genotype interaction with environmental cues influence plant development and metabolic activity in preparation for sustained low temperatures and freezing conditions [50]. A significant level of phenotypic variability and molecular diversity was reported with in-the-wild sugarcane germplasm collected from low temperature regions Lohit and Changlang of Arunachal Pradesh [51]. Moreover, the findings of previous studies with sensitive (*S. officinarum* var CP69-1062) and tolerant (*S. spontaneum*) cultivars that responded differently to cold stress in terms of morphology, physiology and biochemistry when compared to normally grown temperature conditions and to one another indicated different genomic structure, genetic capacity, and their other cold tolerance-related characteristics [52]. Differential expression regulation of genes was also recorded in tolerant genotypes under low temperature conditions compared to room temperature conditions [53–56]. Therefore, yield or biomass is the result of genetic component, physiological and biochemical changes that favor improved performance. Identification of winter tolerant varieties using physiological and biochemical parameters are handy and simple [47]. In most of the cases, many clones or varieties show tolerance to low temperature based on physiological and biochemical traits, but they are poor in biomass accumulation. Hence, we considered mainly the biomass as significant criteria for WTI which is the reflection of genotype and genotype–phenotype interaction including physiological and biochemical changes.

## 5. Conclusions

The wild genetic resources of sugarcane, due to their inherent capacity to withstand climatic vagaries, are of utmost importance for future WT breeding programprograms. The performance of the tropical commercial cultivars (TCC) group was the poorest under a subtropical climate, while the subtropical early (STEM), mid-late (STMM) and *E. arundinaces* introgressed near commercial hybrids (EAIAGH) recorded poor to medium performance, whereas wild species clones and introgressed groups, viz., SSIH, GUC and EASS had better to best performance under an LT regime. The proposed winter tolerance index (WTI), which combines cane yield, winter sprouting and winter growth of plants, classified sugarcane clones according to their winter tolerance behavior. The SSIH (with the best WTI score), EASS and GUC groups were identified as excellent winter ratooner, whereas STEM, STMM and EAIAGH were identified as poor winter ratoon crops, and TCC as winter sensitive. The wild genome introgressed hybrids (AS04-635, AS04-1687, GU07-2276, GU07-3849, AS04-245, AS04-2097, GU07-3774) and commercial cultivar (Co 0238) had excellent/good winter tolerance potential.

## 6. Suggestions and Recommendations

The researchers can use the WTI index for selection of WT genotypes in sugarcane and other crops as it combines cane yield, cane elongation during winter and WSI. As per the index, the genotypes or groups found to have tolerance will have better cane yield potential as well. Co 0238, the commercial variety having good WTI, is already under cultivation. The rest of the better WT genotypes lack desired agronomic and quality attributes, but they can serve as parents for a directed winter tolerance breeding program based on their breeding value.

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