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RELATIVE LEAF WATER CONTENT, PROLINE AND CHLOROPHYLL CONTENT IN RESPONSE TO MOISTURE STRESS AT CRITICAL GROWTH STAGES OF *RABI* MAIZE

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ABSTRACT

Maize (*Zea mays* L.) is highly sensitive to moisture stress, especially during critical phenological stages such as tasseling, silking and grain filling, which significantly influence its physiological functioning and yield. A field experiment was conducted at the University of Agricultural Sciences during *rabi* 2021-22 with nine irrigation treatments replicated thrice to evaluate the effects of moisture stress on physiological parameters such as Relative Leaf Water Content (RLWC), proline accumulation, and chlorophyll content, measured using Soil Plant Analysis Development (SPAD) readings. The results showed that treatments receiving irrigation throughout all crop growth stages recorded significantly higher kernel yield, RLWC and SPAD values compared to those where irrigation was withheld during single stage, dual stage and also during the entire reproductive stage. Conversely, significantly higher proline content was observed in treatments where irrigation was provided only up to the knee-high stage and in those where water was withheld at tasseling, silking, and milky stages, suggesting increased metabolic response to water deficit. Correlation analysis further revealed a strong positive association between kernel yield and both RLWC and SPAD values, and a strong negative association with proline content, indicating that water stress reduces yield potential by impairing physiological processes. These findings emphasize the importance of optimal irrigation scheduling at critical stages to enhance drought tolerance and sustain productivity in *rabi* maize under water-limited conditions.

Key words : Moisture stress, Irrigation scheduling, Relative leaf water content (RLWC), Proline accumulation.

Introduction

Maize (*Zea mays* L.) is an important cereal crop cultivated worldwide and plays a major role in food and feed security. However, water scarcity and drought stress are major constraints to maize production, particularly in regions where irrigation water is limited. In India, water shortage is a significant challenge to sustainable maize production, as a relatively small proportion of the cultivated maize area is irrigated (Sah *et al*., 2020). Water deficit adversely affects maize growth and development, with drought stress during critical reproductive stages causing pronounced reductions in yield and yield components (Sah *et al*., 2020; Hussain *et al*., 2019). Drought stress also induces substantial physiological and biochemical alterations, including reductions in plant water status, photosynthetic activity and nutrient uptake, accompanied by changes in oxidative metabolism (Hussain *et al*., 2019; Zhang *et al*., 2020). Such responses ultimately affect plant growth, reproductive development and grain productivity under water-limited conditions. Therefore, accurate assessment of crop water stress and timely irrigation management are essential for sustaining maize productivity and improving crop resilience to

drought (Seleiman *et al*., 2021; Sheoran *et al*., 2022).

Among the physiological parameters used to assess plant water status under drought conditions, relative water content (RWC) is an important indicator of tissue hydration and plant water relations. RWC reflects the amount of water present in plant tissues relative to their fully hydrated state and is closely associated with the maintenance of cellular hydration and turgor under water-limited conditions (Medici *et al*., 2003; Kim *et al*., 2019). In maize, water-deficit stress can substantially reduce leaf RWC, accompanied by reductions in stomatal conductance, photosynthetic activity and plant growth (Kim *et al*., 2019; Hussain *et al*., 2019). Maintenance of relatively higher RWC under drought indicates a greater capacity of plants to retain water and maintain cellular hydration, which can contribute to the preservation of physiological functions under water stress (Medici *et al*., 2003). Studies in maize have also demonstrated that drought-induced reductions in RWC are associated with physiological responses during critical developmental stages, particularly flowering and grain filling, when water deficit can severely affect reproductive development and yield formation (Kim *et al*., 2019; Sah *et al*., 2020). Therefore, RWC can serve as a useful physiological indicator for evaluating the water status and drought response of maize, particularly when assessing genotypic differences or crop responses to different levels of water availability.

Proline accumulation is an important biochemical response of maize to water-deficit stress. Proline is a compatible osmolyte that can contribute to osmotic adjustment and protection of cellular structures under adverse environmental conditions. Its accumulation under drought has been associated with the maintenance of cellular hydration, protection of proteins and membranes, and regulation of redox balance (Ashraf and Foolad, 2007). In maize, drought stress can increase the accumulation of free proline, together with other stress-responsive metabolites, as part of the plant's physiological and biochemical adjustment to water deficit (Hussain *et al*., 2019; Usmani *et al*., 2020). Yousaf *et al*. (2022) observed increased free-proline accumulation in maize hybrids subjected to drought and combined drought and heat stress, with differences among hybrids indicating variation in their physiological responses to stress. Similarly, Balbaa *et al*. (2022) reported higher proline contents in maize inbred lines under water-stress conditions compared with well-watered conditions, demonstrating the usefulness of proline accumulation as a biochemical response associated with drought stress. Therefore, enhanced proline accumulation can serve as an important physiological and biochemical response for evaluating maize adaptation to water-deficit conditions, although its relationship with drought tolerance may vary among genotypes and stress intensities.

Chlorophyll content, commonly assessed using Soil Plant Development (SPAD) readings, is an important indicator of the physiological status and photosynthetic performance of maize under water-limited conditions. Chlorophyll plays a central role in photosynthesis by absorbing light energy required for carbon assimilation, and reductions in chlorophyll content under water stress can consequently impair photosynthetic activity and crop growth. Recent studies in maize have demonstrated that drought or soil-moisture stress can significantly reduce SPAD values and photosynthetic performance, with the magnitude of reduction depending on the intensity and duration of water stress (Li *et al.*, 2024a; Li *et al.*, 2024b). SPAD measurements provide a rapid and non-destructive means of assessing relative leaf chlorophyll status and have considerable potential for monitoring crop responses to varying water regimes. In particular, SPAD measurements have been successfully evaluated for maize grown under different irrigation levels and growth stages, supporting their application in crop monitoring and precision water management (Ma *et al.*, 2024). Maintenance of higher chlorophyll status under water stress is associated with improved photosynthetic activity, water-use characteristics and dry-matter accumulation, which are important determinants of maize productivity under moisture-limited conditions (Li *et al.*, 2024b).

Materials and Methods

The field experiment was carried out during *rabi* season of 2021-22 at Main Agricultural Research Station, UAS, Dharwad, Karnataka, India. The geographical co-ordinates of experimental site is 15º 29' North latitude and 74º 59' East longitude at an altitude of 678 m above mean sea level. UAS, Dharwad comes under Northern Transitional Zone (Zone-8) of Karnataka. Maize hybrid NK-6240 with spacing of 60 × 20 cm was used for the investigation. During the crop growing period (10th December 2021 – 8th April 2022) a total rainfall of 57.2 mm was received in the last two weeks of March (48.8 mm) and first two weeks of April (8.4 mm). There was no influence of rains over skipping of irrigation at critical stages and no deviation in treatment imposition. The soil of the experimental site was medium deep black soil, slightly alkaline (pH 7.8), with normal electrical conductivity (0.32 dS m^{-1}), low in organic carbon content (0.46%), low in available nitrogen (278.00 kg N ha^{-1}), medium in available phosphorus (35.00 kg P_2O_5 ha^{-1}) and

high in potassium (393.00 kg K_2O ha^{-1}).

The experiment was laid out in randomized complete block design with nine treatments and three replications (Plate 1). Treatment comprises of (T_1) Irrigations at all growth stages (VE, V4, V8, VT, R1, R3 and R4), (T_2) skipping irrigation only at tasseling (VT), (T_3) skipping irrigation only at silking (R1), (T_4) skipping irrigation only at milky stage (R3), (T_5) skipping irrigation at tasseling and silking (VT and R1), (T_6) skipping irrigation at tasseling and milky stage (VT and R3), (T_7) skipping irrigation at silking and milky stage (R1 and R3), (T_8) skipping irrigations at all critical stages (tasseling, silking and milky stage) and (T_9) irrigations upto knee high stage (V8) only.

Fertilizers were applied at the rate of 150: 65: 65 N: P_2O_5: K_2O kg ha^{-1} in the form of urea (N), Di-Ammonium phosphate (DAP) and Muriate of potash (K_2O). At the time of sowing, 50% of N and 100 % of P_2O_5 and K_2O were applied as basal dose followed by 50% of N applied at 35 days after sowing. Irrigation was provided after sowing (VE) to ensure uniform germination and establishment of the crop. Later irrigation was scheduled at four leaf stage (V4) and knee high stage (V8), for all the treatments. Subsequent irrigations were scheduled as per the treatments at tasseling stage (VT), silking stage (R1), milky stage (R3) and dough stage (R4). Irrigation was provided to each plot with 2 inches' pipe (siphon) at a discharge rate of 2.5 liters sec^{-1}. Care was taken to minimize horizontal movement of water to other treatments.

Observations recorded

Relative Leaf Water content

Relative leaf water content (RLWC) was estimated by following the procedure as outlined by Barrs and Weatherly (1962) at VT, R1, R3 and R4 stages. Twenty leaf discs of third fully expanded leaf from the top were collected and weighed on an electronic balance and fresh weight was determined. The weighed leaf discs were floated in a petridish containing distilled water for four hours and then wiped using blotting paper gently and weight was taken again, which was referred to as the turgid weight. The leaf discs were oven dried at 80ºC for 48 hours and dry weight was recorded. The RLWC was calculated by using the following formula and expressed in percentage.

$$\text{RLWC (\%)} = \frac{\text{Fresh weight (g)} - \text{Dry weight (g)}}{\text{Turgid weight (g)} - \text{Dry weight(g)}} \times 100$$

Proline determination

Proline content was estimated as per the procedure outlined by Bates *et al.* (1973). It was expressed in micro mole per gram fresh weight of leaf (µ mol g FW^{-1}). Observations recorded at R1 and R4 stages of the crop.

$$\text{Proline (µ mol g FW}^{-1}) = \frac{34.11 \times OD_{520} \times V}{2 \times f} \times 100$$

Where,

OD_{520} = Optical density/absorbance at 520 nm

V = Total volume of extract (mL)

f = Fresh weight of leaf sample (g)

2 = Volume of extract used for the reaction (mL)

100 = Conversion factor

34.11 = Proline concentration factor obtained from the standard curve

Measuring chlorophyll content using SPAD meter

SPAD meter is a simple, portable proximal sensor that measures the relative chlorophyll content of leaves. The SPAD meter measures the difference between the transmittance of a red (650 nm) and an infrared (940 nm) light through the leaf. Before taking the readings the meter was calibrated by switching on the meter without leaf in the sample slot. After calibration, selected leaf was inserted into the sample slot of measuring head of SPAD meter. SPAD values displayed on the screen are noted. Observations recorded at VT, R1, R3 and R4 stage.

Results and Discussion

Relative Leaf Water content

Relative leaf water content (RLWC) is an important indicator of the water status of plants and reflects the degree of cellular hydration and water retention capacity of leaf tissues. It is strongly influenced by soil moisture availability and the prevailing irrigation regime. In the present study, RLWC showed a declining trend with the advancement of crop growth from the tasseling to dough stage, particularly under treatments exposed to prolonged moisture stress (Table 1).

At the tasseling stage, no significant differences in RLWC were observed among the irrigation treatments, indicating that the plants maintained relatively stable leaf water status during the initial phase of reproductive development. However, significant differences became evident at subsequent growth stages. Irrigation scheduled at all crop growth stages recorded significantly higher RLWC at R1 (79.7%), R3 (78.3%) and R4 (73.8%) stages. In contrast, irrigation provided only up to the knee-high stage resulted in significantly lower RLWC at the silking (70.1%), milking (64.8%) and dough (59.1%) stages. Treatments in which irrigation was skipped at all

Table 1 : Relative leaf water content (%) as influenced by moisture stress at critical crop growth stages.

Treatments	**VT (Tasseling stage)**	**R1 (Silking stage)**	**R3 (Milky stage)**	**R4 (Dough stage)**
T_1:Irrigations at VE, V4, V8, VT, R1, R3 and R4	80.6^{a}	79.7^{a}	78.3^{a}	73.8^{a}
T_2:Irrigations at VE, V4, V8, R1, R3 and R4 (Skipping VT)	74.7^{a}	73.7^{bc}	73.5^{ab}	70.2^{ab}
T_3:Irrigations at VE, V4, V8, VT, R3 and R4 (Skipping R1)	79.5^{a}	74.9^{b}	73.8^{ab}	70.5^{ab}
T_4:Irrigations at VE, V4, V8, VT, R1 and R4 (Skipping R3)	80.0^{a}	79.4^{a}	76.4^{a}	71.8^{a}
T_5:Irrigations at VE, V4, V8, R3 and R4 (Skipping VT and R1)	74.5^{a}	70.6^{c}	68.8^{bc}	62.4^{cd}
T_6:Irrigations at VE, V4, V8, R1 and R4 (Skipping VT and R3)	74.7^{a}	73.8^{bc}	71.2^{b}	66.7^{bc}
T_7:Irrigations at VE, V4, V8, VT and R4 (Skipping R1 and R3)	78.3^{a}	74.7^{b}	71.0^{b}	65.2^{c}
T_8:Irrigations at VE,V4,V8 and R4 (Skipping VT, R1 and R3)	74.6^{a}	70.5^{c}	65.5^{c}	59.6^{d}
T_9: Irrigations at VE, V4 and V8. (Skipping VT, R1, R3 and R4)	74.5^{a}	70.1^{c}	64.8^{c}	59.1^{d}
S.Em.±	2.0	1.3	1.5	1.5

Note: **VE** = Emergence stage, **V4 =** four leaves visible with collar (15-20 DAS), **V8 =** Knee high stage (30-35DAS), **VT**=Tasseling stage (50-55 DAS), **R1 =** Silking stage (60-65 DAS), **R3 =** Milky stage (75-80 DAS) and **R4 =** Dough stage (90-95 DAS).

critical stages or at both tasseling and silking stages recorded RLWC values statistically comparable with the treatment irrigated only up to the knee-high stage (Table 1).

The progressive decline in RLWC under prolonged irrigation deficit may be attributed to continuous depletion of soil moisture in the root zone, which restricted water uptake and consequently reduced leaf hydration. When soil moisture becomes limiting, plants progressively lose cellular water, resulting in reduced cell turgidity and lower relative leaf water content. The effect becomes more pronounced when irrigation is withheld across two or more successive critical growth stages, as the prolonged water deficit may cause soil moisture to decline towards or below the permanent wilting range. Similar reductions in plant water status under inadequate irrigation have been reported in maize. Adamu *et al*. (2014) observed differences in physiological responses and water-use characteristics of maize hybrids subjected to different irrigation regimes, demonstrating the influence of irrigation availability on plant water relations. Similarly, Renuka (2020) reported variation in drought response among maize genotypes under water stress imposed through a line-source sprinkler irrigation system, indicating the importance of water availability in maintaining physiological performance of maize.

Treatments in which irrigation was skipped at only one critical growth stage but resumed during the subsequent stages showed an improvement in RLWC at the milking and dough stages (Table 1). The recovery in RLWC following rewatering can be attributed to the restoration of soil moisture in the root zone, which facilitated renewed water uptake and improved leaf hydration. Rewatering enables plants to restore tissue water content and recover, at least partially, from drought-induced reductions in physiological activity. Liu *et al*. (2024) demonstrated that maize leaves can recover their physiological functions following rewatering after drought stress; however, the extent of recovery depends on the severity and duration of the preceding drought period. Thus, the recovery observed in the present study following a single skipped irrigation event suggests that temporary water stress was partially reversible when adequate moisture was restored during the subsequent growth stage.

A pronounced reduction in RLWC from tasseling to dough stage was observed in the treatments where irrigation was skipped at all critical stages and where irrigation was provided only up to the knee-high stage. In the treatment with irrigation skipped at all critical stages, RLWC declined from 74.5% at tasseling to 59.1% at the dough stage, while in the treatment irrigated only up to the knee-high stage, it declined from 74.6% to 59.6%. The substantial decline in these treatments indicates the cumulative effect of prolonged soil moisture depletion on plant water status. As the crop progressed towards maturity, increasing atmospheric demand combined with restricted soil moisture availability likely intensified water loss from leaves, resulting in reduced cellular hydration and RLWC.

The greater reduction in RLWC at the milking and dough stages is particularly important because these stages represent periods of high assimilate demand and continued reproductive development in maize. Inadequate water availability during these stages can impair leaf hydration and physiological activity, thereby potentially

affecting photosynthetic efficiency and assimilate translocation to developing grains. Conversely, maintaining irrigation throughout the critical growth stages helped sustain higher RLWC, indicating better plant water status and improved maintenance of cellular turgidity.

Overall, the results demonstrate that the maintenance of adequate soil moisture through irrigation at critical growth stages is essential for sustaining leaf water status in maize. A single interruption in irrigation followed by timely rewatering allowed partial recovery of RLWC, whereas continuous or repeated omission of irrigation resulted in a progressive and substantial decline in leaf water content. These findings are consistent with the observations of Adamu *et al*. (2014), Renuka (2020) and Liu *et al*. (2024) and indicate that the ability of maize plants to maintain or recover leaf water status depends strongly on the duration and severity of water deficit and the availability of moisture during subsequent crop growth stages.

Proline accumulation is an important biochemical response of plants to moisture stress and is considered a useful indicator of the severity of water deficit. In the present study, proline content varied significantly among the irrigation treatments, with greater accumulation observed under moisture-stressed conditions (Table 2). Irrigation provided only up to the knee-high stage recorded significantly higher proline content at both silking (9.09 µmol g^{-1} FW) and dough (13.37 µmol g^{-1} FW) stages, and these values were statistically at par with the treatment in which irrigation was skipped at all critical stages. In contrast, significantly lower proline content was recorded under irrigation provided at all crop growth stages, with 3.56 µmol g^{-1} FW at silking and 4.02 µmol g^{-1} FW at dough stage.

The higher proline accumulation under restricted irrigation may be attributed to the development of water deficit in the crop root zone. Under moisture stress, plants accumulate compatible solutes such as proline as part of their adaptive mechanism to maintain cellular water balance. Proline contributes to osmotic adjustment by lowering cellular osmotic potential, thereby facilitating water retention and helping to maintain cell turgidity under declining soil moisture conditions. It may also provide protection to cellular membranes and proteins during stress. Therefore, the higher proline concentration observed under restricted irrigation in the present study indicates greater physiological stress experienced by the plants.

A progressive increase in proline content from silking to dough stage was evident under prolonged moisture stress. In the treatment irrigated only up to the knee-high stage, proline content increased from 9.09 to 13.37 µmol g^{-1} FW, indicating an intensification of water stress with advancement of the crop towards maturity. This increase may be associated with progressive depletion of soil moisture and increased crop water demand during the reproductive and grain-filling stages. Ghahfarokhi *et al*. (2015) reported increased proline accumulation in maize hybrids under drought stress at different growth stages and observed changes in relative water content and other physiological parameters following drought and rewatering. A similar increase in proline accumulation under drought conditions has been reported in maize by Efeoglu *et al*. (2009), who observed enhanced proline accumulation in maize cultivars subjected to drought stress.

The lower proline content recorded under irrigation at all crop growth stages indicates that adequate water availability minimized the need for stress-induced osmotic adjustment. Maintenance of sufficient soil moisture likely supported continuous water uptake and better tissue hydration, thereby reducing the physiological stress experienced by the plants. In contrast, repeated or prolonged withholding of irrigation resulted in greater water deficit and consequently stimulated higher proline accumulation. Renuka (2020), while screening maize genotypes for drought tolerance using a line-source sprinkler irrigation technique, also demonstrated considerable variation in the physiological response of maize under different levels of water availability, emphasizing the importance of irrigation management in determining drought response. The present findings are consistent with the role of metabolite-mediated responses in crop adaptation to drought. Zhang *et al*. (2024) reported that drought stress induces metabolic adjustments that contribute to the adaptation and tolerance of crops under limited water availability. In the present study, greater proline accumulation was observed under moisture-stressed conditions, particularly in treatments subjected to prolonged water deficit. This response may reflect an adaptive adjustment of maize plants to declining water availability, as proline accumulation is commonly associated with osmotic adjustment and stress protection.

The pattern of proline accumulation also corresponded with the relative leaf water content results. Treatments experiencing prolonged moisture stress showed lower relative leaf water content together with higher proline accumulation, indicating an association between declining plant water status and increased proline accumulation. In contrast, adequately irrigated treatments maintained higher leaf water content and comparatively

Table 2 : Proline content (µ mol g FW^{-1}) as influenced by moisture stress at critical crop growth stages.

Treatments	R1 (Silking stage)	R4 (Dough stage)
T_1: Irrigations at VE, V4, V8, VT, R1, R3 and R4	3.56^{c}	4.02^{e}
T_2:Irrigations at VE, V4, V8, R1, R3 and R4 (Skipping VT)	5.86^{b}	6.39^{d}
T_3:Irrigations at VE, V4, V8, VT, R3 and R4 (Skipping R1)	5.89^{b}	6.35^{d}
T_4:Irrigations at VE, V4, V8, VT, R1 and R4 (Skipping R3)	3.55^{c}	5.36^{de}
T_5:Irrigations at VE, V4, V8, R3 and R4 (Skipping VT and R1)	8.64^{a}	10.88^{b}
T_6:Irrigations at VE, V4, V8, R1 and R4 (Skipping VT and R3)	6.09^{b}	8.95^{c}
T_7:Irrigations at VE, V4, V8, VT and R4 (Skipping R1 and R3)	6.11^{b}	9.21^{c}
T_8:Irrigations at VE,V4,V8 and R4 (Skipping VT, R1 and R3)	8.98^{a}	12.98^{a}
T_9: Irrigations at VE, V4 and V8. (Skipping VT, R1, R3 and R4)	9.09^{a}	13.37^{a}
S.Em.±	0.38	0.49

Note: **VE** = Emergence stage, **V4 =** four leaves visible with collar (15-20 DAS), **V8 =** Knee high stage (30-35DAS), **VT=** Tasseling stage (50-55 DAS), **R1 =** Silking stage (60-65 DAS), **R3 =** Milky stage (75-80 DAS) and **R4 =** Dough stage (90-95 DAS).

lower proline levels. Thus, the observed increase in proline under moisture deficit may represent a physiological response associated with the adjustment of maize plants to water stress.

SPAD value is indirect indicator of relative chlorophyll content. SPAD values were decreased with the progress of the crop growth i.e. from tasseling to silking in all the treatments. This is mainly due to decreased greenness of plant approaching towards maturity (Table 3). Significantly higher SPAD values in the treatment with irrigations at all stages were recorded at silking (44.3), at milky stage (40.9) and at dough stage (38.3) over the rest of the treatments. This is mainly due to higher rate of photosynthesis and higher chlorophyll content. These results are in harmony with the findings of Guang-Hua Yin *et al*. (2012), who reported significantly higher chlorophyll content under well watered conditions compared to moisture stressed condition. However, it was on par with irrigations skipped only at tasseling, only at silking and only at milky stage during dough stage (Table 3). This was mainly due to recovery in rate of photosynthesis and chlorophyll content by providing irrigation at subsequent stages.

Significantly lower SPAD values were recorded with irrigations up to knee high stage at silking (34.2), at milky (30.2) and at harvest (27.2). Decrease in SPAD values from 34.2 to 30.2 was recorded with moisture stressed situation from knee high stage. This was mainly due to reduced photosynthetic rate, reduced chlorophyll content and drying of leaves under moisture stress condition. These results are harmonious with findings of Adamu *et al*. (2014) who reported decrease in SPAD values from 41.29 to 29.77 under moisture stress condition.

Applying irrigation throughout all growth stages of the crop resulted in the highest kernel yield (7040.7 kg ha^{-1}), significantly surpassing all other treatments (Table 4). These results are consistent with the observations of Gouranga and Verma (2005), who reported a yield of 5187 kg ha^{-1} under conditions without any moisture stress during the tasseling, silking, and grain-filling stages. In contrast, skipping irrigation at the tasseling or silking stage alone led to notable reductions in yield, recording 5744.7 kg ha^{-1} and 5874.7 kg ha^{-1}, respectively (Table 4). A more pronounced decline was observed when irrigation was omitted during both the tasseling and silking stages, with yields dropping to 3681.3 kg ha^{-1}, which was significantly lower than when irrigation was skipped at either stage individually. The lowest yield (2628.3 kg ha^{-1}) was recorded when irrigation was provided only up to the knee-high stage. This outcome was statistically comparable to the treatment where irrigation was withheld at the tasseling, silking, and milky stages, which yielded 2729.7 kg ha^{-1} (Table 4).

A significant reduction in kernel yield (62.7%) was observed when irrigation was applied only up to the knee-high stage. The next highest reduction (61.2%) occurred when irrigation was withheld at all critical growth stages. This yield loss was primarily due to poor fertilization efficiency and disrupted carbohydrate translocation from source to sink, caused by insufficient soil moisture in the root zone. This resulted in smaller cobs with fewer filled kernels, ultimately leading to lower yields. Among the various dual-stage irrigation omission treatments, the greatest yield decline (47.7%) occurred when irrigation was skipped during both the tasseling and silking stages. These two stages are crucial in determining the number of kernels per cob, and water stress during this period led to severe kernel abortion. These findings align with those of Yildirim *et al*. (1996), who reported a 25.2% yield reduction under similar conditions (Table 4). When

Table 3 : SPAD values as influenced by moisture stress at critical crop growth stages.

Treatments	VT (Tasseling stage)	R1 (Silking stage)	R3 (Milky stage)	R4 (Dough stage)
T_1: Irrigations at VE, V4, V8, VT, R1, R3 and R4	45.8^{a}	44.3^{a}	40.9^{a}	38.3^{a}
T_2:Irrigations at VE, V4, V8, R1, R3 and R4 (Skipping VT)	42.6^{a}	41.2^{b}	38.4^{ab}	36.0^{ab}
T_3:Irrigations at VE, V4, V8, VT, R3 and R4 (Skipping R1)	45.6^{a}	41.3^{b}	38.9^{ab}	36.3^{ab}
T_4:Irrigations at VE, V4, V8, VT, R1 and R4 (Skipping R3)	45.2^{a}	44.2^{a}	39.7^{a}	37.0^{a}
T_5:Irrigations at VE, V4, V8, R3 and R4 (Skipping VT and R1)	42.3^{a}	35.6^{c}	33.7^{c}	32.0^{c}
T_6:Irrigations at VE, V4, V8, R1 and R4 (Skipping VT and R3)	42.3^{a}	39.9^{b}	36.1^{bc}	33.5^{bc}
T_7:Irrigations at VE, V4, V8, VT and R4 (Skipping R1 and R3)	45.7^{a}	40.8^{b}	35.9^{bc}	33.3^{bc}
T_8:Irrigations at VE,V4,V8 and R4 (Skipping VT, R1 and R3)	42.4^{a}	35.0^{c}	30.6^{d}	28.3^{d}
T_9: Irrigations at VE, V4 and V8. (Skipping VT, R1, R3 and R4)	42.2^{a}	34.2^{c}	30.2^{d}	27.2^{d}
S.Em.±	1.4	0.9	1.0	1.1

Note: **VE** = Emergence stage, **V4 =** four leaves visible with collar (15-20 DAS), **V8 =** Knee high stage (30-35 DAS), **VT =** Tasseling stage (50-55 DAS), **R1 =** Silking stage (60-65 DAS), **R3 =** Milky stage (75-80 DAS) and **R4 =** Dough stage (90-95 DAS).

Table 4 : Yield of maize as influenced by moisture stress at critical crop growth stages.

Treatment details	Kernel yield (kg ha^{-1})	Reduction in kernel yield (%)
T_1: Irrigations at VE, V4, V8, VT, R1, R3 and R4	7040.7^{a}	-
T_2:Irrigations at VE, V4, V8, R1, R3 and R4 (Skipping VT)	5744.7^{c}	18.4
T_3:Irrigations at VE, V4, V8, VT, R3 and R4 (Skipping R1)	5874.7^{c}	16.6
T_4:Irrigations at VE, V4, V8, VT, R1 and R4 (Skipping R3)	6411.0^{b}	9.0
T_5:Irrigations at VE, V4, V8, R3 and R4 (Skipping VT and R1)	3681.3^{e}	47.7
T_6:Irrigations at VE, V4, V8, R1 and R4 (Skipping VT and R3)	4271.3^{d}	39.3
T_7:Irrigations at VE, V4, V8, VT and R4 (Skipping R1 and R3)	4016.0^{de}	43.0
T_8:Irrigations at VE,V4,V8 and R4 (Skipping VT, R1 and R3)	2729.7^{f}	61.2
T_9: Irrigations at VE, V4 and V8. (Skipping VT, R1, R3 and R4)	2628.3^{f}	62.7
S.Em.±	171.6	-

Note: **VE** = Emergence stage, **V4 =** four leaves visible with collar (15-20 DAS), **V8 =** Knee high stage (30-35DAS), **VT =** Tasseling stage (50-55 DAS), **R1 =** Silking stage (60-65 DAS), **R3 =** Milky stage (75-80 DAS) and **R4 =** Dough stage (90-95 DAS).

water stress was imposed at the tasseling and milky stages, despite adequate irrigation at silking, pollen viability remained relatively unaffected. However, limited water availability at the milky stage disrupted the distribution of stored carbohydrates, causing uneven grain development, smaller kernel size, and poorly filled cob tips. Furthermore, when stress occurred during silking and milky stages, fertilization was hindered due to non-receptive stigmas and ineffective pollen transfer, resulting in fewer and deformed grains per cob. These results are supported by the findings of Cakir (2004), who reported yield losses ranging from 66% to 93% when irrigation was skipped during both tasseling and cob formation. Among treatments where irrigation was skipped at only one stage, withholding water at the tasseling stage led to the highest yield reduction (18.4%) compared to other single-stage omissions (Table 4).

The correlation heat map (Fig. 1) revealed strong associations between kernel yield and key physiological traits in maize under moisture stress conditions. Kernel yield exhibited strong negative correlations with proline content at the R1 and R4 stages, indicating greater proline accumulation under increased moisture stress and its association with reduced yield. In contrast, SPAD values showed positive correlations with kernel yield across the evaluated growth stages, with stronger associations during the reproductive and grain-filling stages, indicating an association between higher chlorophyll status and better yield performance. Similarly, relative leaf water content at VT, R1, R3 and R4 stages showed positive correlations with kernel yield, highlighting the importance of maintaining favourable plant water status throughout the reproductive and grain-filling periods.

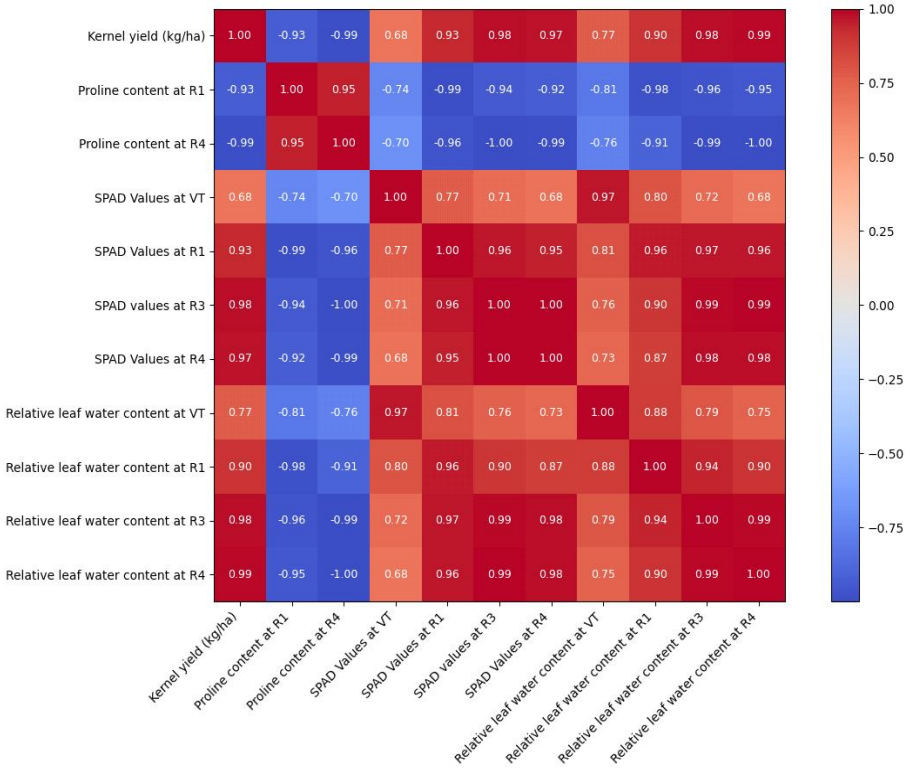


Fig. 1 : Correlation heat map.

Conclusion

Irrigation throughout the critical growth stages improved kernel yield and helped maintain physiological functions in *rabi* maize. Moisture stress imposed at critical stages, particularly tasseling, silking and milky stages, resulted in substantial reductions in kernel yield and was associated with lower SPAD values and relative leaf water content (RLWC), along with increased proline accumulation, indicating greater physiological stress. The greatest yield reduction was observed when irrigation was restricted to the knee-high stage, while omission of irrigation at individual reproductive stages, particularly tasseling and silking, also resulted in marked yield losses. Correlation analysis further showed that kernel yield was positively associated with SPAD values and RLWC, whereas proline content showed a negative association with yield. These findings highlight the importance of maintaining adequate soil moisture during the reproductive and grain-filling stages to sustain favourable plant water status and physiological activity and thereby support kernel development and yield formation. Ensuring irrigation at critical growth stages can therefore contribute to improved maize productivity, particularly in drought-prone environments where water availability is limited.

Conflict of interest

Authors declare no conflict of interest.

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