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# Assessment of Total Crude Protein during Growth and Development of Some Sorghum (*Sorghum bicolor (*L.*)* Moench.) Cultivars Grown under Water Stress*

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**Abstract:** The total crude protein (TCP) changes during growth and development of some sorghum (*Sorghum bicolor (*L.*)* Moench) cultivars under water stress was studied in a field experiment for two seasons (2017/2018 and 2018/2019) at the Demonstration Farm, Faculty of Agriculture, University of Khartoum. The design was split plot with three replicates. The main plots were assigned to water treatments and the subplots to the cultivars. Water stress was induced by watering treatments at intervals of 7 (T1), 14 (T2) and 21 (T3) days, and the sorghum cultivars were Wad Ahmed, Arfa Gadmak, Tabbat, Abu sabien and White Mugud**.** Leaves were collected for analysis of total crude protein percentage; at different growth stages (seedling, panicle initiation, booting, flowering, milk, soft dough, hard dough and physiological maturity). The results showed differences in TCP percentage among the cultivars with the highest value (15.2 %) recorded for Wad Ahmed under non-sress and the lowest values (8.4 %) recorded for White Mugud under non-stress Contrary to the other four varieties, TCP of Abu Sabien decreased from

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13.8 % under non-stress to 10.6 % under stress conditions. The TCP percentage gradually decreased towards booting and flowering stages in most cultivars and they produced a high TCP percentage under the long watering interval (21days).

**Keywords:** Total crude protein, sorghum cultivar, water stress

## INTRODUCTION

*Sorghum bicolor* is a grass species cultivated for grain and straw, used for food, both for animals and humans, and for ethanol production. Sorghum originated in northern Africa, and is now cultivated widely in tropical and subtropical regions. It is the world's fifth most important cereal crop after rice, wheat, maize and barley. Sorghum is one of the most important cereals, representing a major source of energy and protein for millions of people, especially in Africa and for livestock worldwide (Reddy *et al*., 2009; Ashok-Kumar *et al*., 2010). Sorghum grain contains on average per 100 g edible portion: 9.2 g. water, 1418 kJ (339 kcal) energy, 11.3 g. protein , 3.3 g. fat, 74.6 g. carbohydrate . (Stenhouse and TippayarukL, 1996). Sorghum proteins are located in the endosperm (80%), germ (16%) and pericarp (3%) (Taylor and Schussle, 1986). Nitrogen fertilization significantly increases prolamins or kafirin accumulation and protein content (Warsi and Wright, 1973).

Drought is one of the most important abiotic stresses limiting sorghum productivity around the world (Nguyen, 2008). It is certainly of great significance in the semi-arid tropics (SAT), where rainfall is generally low and its distribution is erratic (Ejeta *et al*., 1999). Decreased water availability and increased food demand worldwide require development of more water-efficient crops (Balota *et al*., 2008). Sorghum is one of the crops that tolerate drought in stressed environments (Saxena and O'Toole, 2002). In sorghum genes located in four genomic regions (Stg1, Stg2, Stg3 and Stg4) control the functional basis of the Parental source of 'stay-green' (Borrell *et al*., 2008). Sudan, and particularly Darfur, has a large and diverse sorghum germplasm which is, usually, subjected to both water and heat stresses especially during drought. This germplasm may prove to be a valuable source of proteins and can be utilized to develop cultivars with high protein content under drought conditions.

The objective of this study, therefore, was assessment of total crude protein during growth and development of some sorghum (*Sorghum bicolor (*L.*) Monech*) cultivars under water stress.

## MATERIALS AND METHOD*S*

### Field Experiments

A split-plot design with three replications was used, with random assignment of watering treatments to the main plots and sorghum cultivars to the sub-plots. Seeds were sown on September, 25 in 2017 and on September, 1 in 2018. Land preparation was done by disc harrowing, followed by leveling and then ridging.

Seeds were sown at a rate of 5 seeds per hole then were later thinned to three plants per hole after two weeks. Spacing was 25cm between holes and 80cm between ridges. Each plot contained four ridges of 3m length each.

Plants were watered every 7 days during the establishment phase, which continued for four weeks, to ensure uniform germination and crop establishment. Then water stress was imposed by withholding irrigation water from the plots every 14 or every 21 days, while the control plots were regularly irrigated every seven days.

### Protein analysis

Leaf blade samples were collected randomly from each genotype from each plot to extract proteins at the following stages of growth: seedling stage, panicle initiation, booting, 50% flowering, milk stage, soft dough, hard dough and physiological maturity. Also heads were collected for protein extraction to study late Embryogenesis Abundant (LEA) proteins.
Leaves were dried in an oven at 80°C for 48h.The dry leaves were ground in mortar and pestle and further used for protein estimation. Crude protein was determined according to the method of AOAC (1975) using micro-

Kjeldahl nitrogen digestion and distillation method. A sample of 0.2 g of oven dried leaves was weighed and put into 100ml kjeldahl flask then one tablet catalyst and 3.5 ml of concentrated sulphuric acid were added. The samples and contents were heated on an electric heater for 2h. The sample was cooled, diluted, and was placed in the distillation apparatus, 20ml of 40% NaOH was added and distilled for 7 min. The ammonia evolved was received in 10ml of 21 boric acid solution, contained in a conical flask attached to the receiving end. The trapped ammonia was titrated against 0.02 HCL using universal indicator (methyl red + bromocresol green). Nitrogen and then protein contents were calculated as follows:

$$N\% = \frac{\text{Volume of HCl x 0.02 x14}}{\text{Sample weight x 1000}} x100$$

$$\text{Protein \%} = \text{N \% x 6.25}$$

## RESULT

**First Season**

**Total Crude Protein % of Wad Ahmed Cultivar**

Figure 1 shows the variation in TCP% of Wad Ahmed during different stages of growth in the first season. TCP% was observed to progressively decline from the seedling stage reaching its lowest value (9.36, 8.62 and 9.88% in 7, 14 and 21 days respectively) in the flowering stage. Thereafter, it began to increase progressively reaching its highest value (13.50, 14.00 and 15.20% in 7, 14 and 21 days respectively) in the physiological maturity stage. Significant differences were observed in seedling, hard dough and physiological maturity stages.

**Total Crude Protein % of Arfa Gadamk Cultivar**

Figure 2 represents the change in TCP% of Arfa Gadamk during different stages of growth in the first season. The TCP% gradually reduced during growth, the highest reduction (10.53, 8.77 and 11.7% in 7, 14 and 21 days, respectively) was observed in soft dough stage .Water stress significantly increased the TCP in hard dough stage, where the long watering intervals of 21 days produced a high TCP (15.80%).

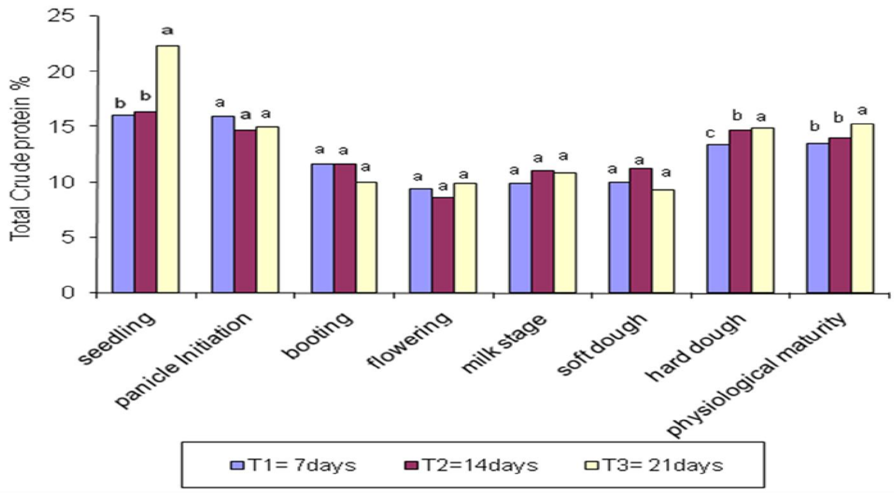


Fig. 1. TCP% of Wad Ahmed during different stages of growth (Season I)
*Comparisons were done within each growth stages individually at $p \leq 0.05$ level of significant

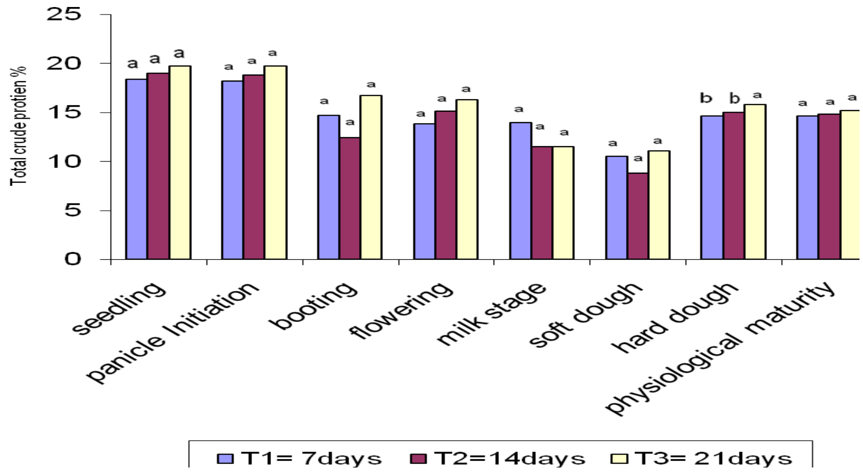


Fig. 2**.** TCP% of Arfa Gadamk during different stages of growth (in season 1).
*Comparisons were done within each growth stages individually at $p \leq 0.05$ level of significant

**Total Crude Protein % of Tabbat Cultivar**

Figure 3 reveals the variation in TCP% of Tabbat during different stages of growth in the first season. TCP% was observed to progressively decline from the seedling stage reaching its lowest value (11.1, 12.01 and 12.15% in 7, 14 and 21 days respectively) in the flowering stage. Thereafter, it began to increase progressively reaching its highest value (14.00, 14.80 and 15.20% in 7, 14 and 21 days respectively) in the physiological maturity stage. Water treatments significantly affected the TCP% during seedling, milk, soft dough, hard dough, and physiological maturity stages. Irrigation every 7 days yielded a high TCP% in seedling stage (20.91%), while watering every 21 days gave the highest TCP in milk (16.67%), hard dough (15.80%) and physiological maturity stages (15.20%). At soft dough stage watering intervals of 14 days gave the lowest TCP%.

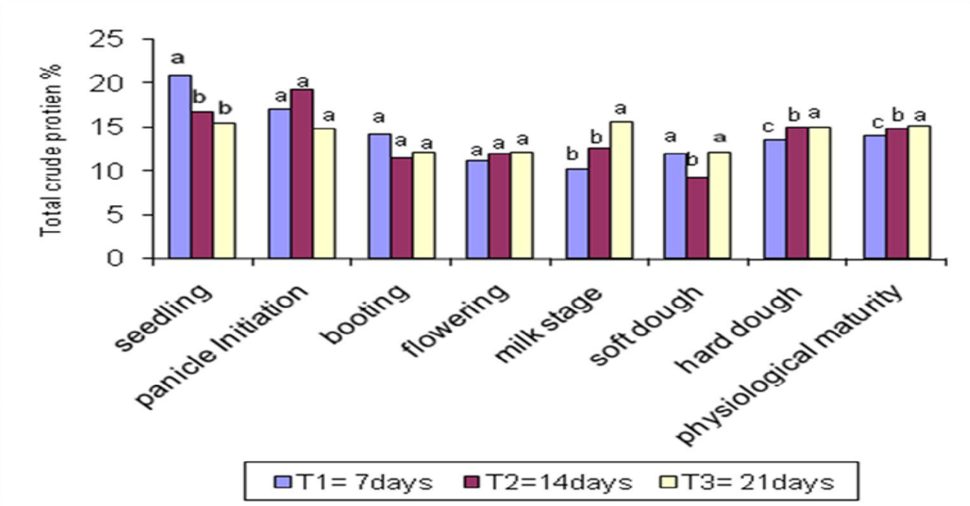


Fig. 3. TCP% of Tabbat during different stages of growth (Season I)
*Comparisons were done within each growth stages individually at $p \leq 0.05$level of significant

**Total Crude Protein % of Abu Sabien Cultivar**

Figure 4 reflects the TCP% content of Abu Sabien under water stress during different growth stages in the first season. TCP% was recognized to progressively decline from the seedling stage reaching its lowest value (13.78, 8.48 and 10.58% in 7, 14 and 21 days respectively) in the booting stage and did not increase again. Significant differences were recorded in booting stage, where the short watering interval of 7days produced the high TCP (13.78%).

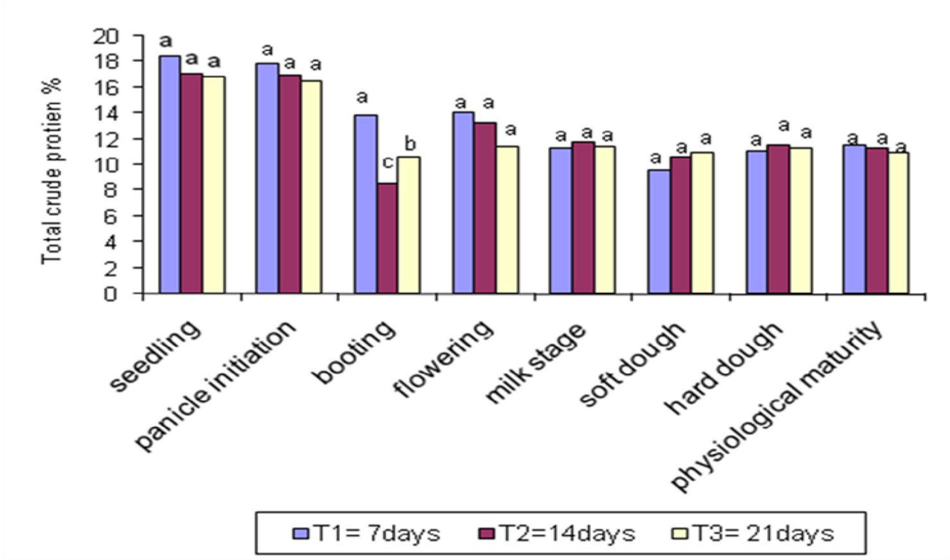


Fig. 4. TCP% of Abu Sabien during different stages of growth (Season I) *Comparisons were done within each growth stages individually at $p \leq 0.05$ level of significant

**Total Crude Protein % of White Mugud Cultivar**

The variations in TCP% of White Mugud during different stages of growth in the first season are presented in Figure 5. A gradual decline was observed from seedling stage reaching its lowest value (8.41, 8.66 and 11.72% in 7, 14 and 21 days respectively) in the booting and soft dough (8.97, 8.17 and 12.73% in 7, 14 and 21 days respectively) stages, thereafter it began to rise, reaching its highest value (14.80, 15.20 and 15.40% in 7, 14 and 21 days, respectively) in physiological maturity stage. Significant differences were registered among booting, flowering, soft dough and hard dough stages, where the long watering intervals of 21days produced a high TCP%.

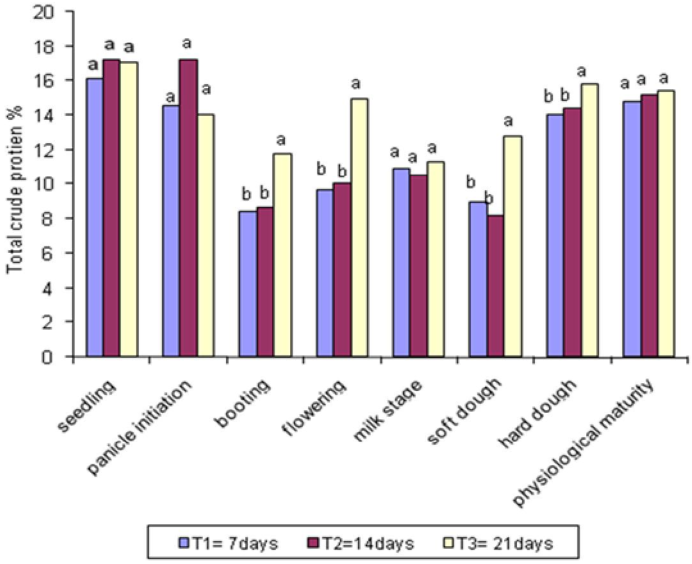


Fig.5. TCP% of White Mugud during different stages of growth (SeasonI) *Comparisons were done within each growth stages individually at p ≤ 0.05 level of significant

**Second Season**

**Total Crude Protein % of Wad Ahmed Cultivar**

The variations in TCP% of Wad Ahmed during different stages of growth in the second season are presented in Figure 6. TCP% was observed to decline from the seedling stage reaching its lowest value (10.17, 10.52 and 11.11% in 7, 14 and 21 days respectively) in the booting stage. It began to increase reaching its highest value in milk (14.93, 16.24 and 16.40% in 7, 14 and 21 days respectively) and soft dough (14.21, 15.29 and 16.12% in 7, 14 and 21 days respectively) stages. On the other hand, the long watering intervals of 21 days produced a high TCP% during panicle initiation (16.60%), milk (16.40%), soft dough (16.12%) and physiological maturity (15.17%) stages.

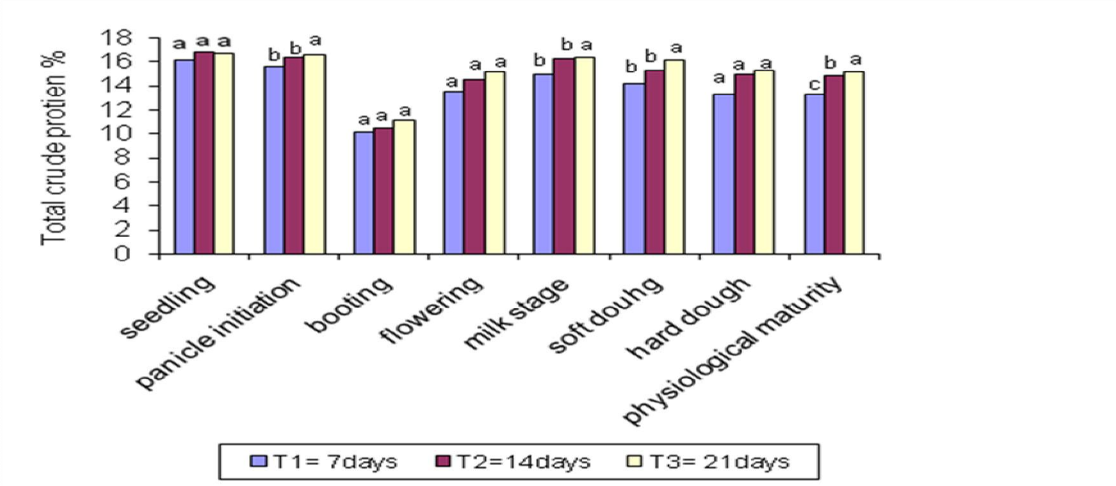


Fig.6. TCP% of Wad Ahmed during different stages of growth (Season II) *Comparisons were done within each growth stages individually at $p \leq 0.05$ level of significant

**Total Crude Protein % of Arfa Gadamk Cultivar**

The change in TCP% of Arfa Gadamk during different stages of growth in the second season is presented in Figure 7. A clear reduction of TCP% was observed in booting stage (10.53, 11.29 and 11.15% in 7, 14 and 21 days respectively) after the initial high levels in seedling (15.20, 16.20 and 16.40% in 7, 14 and 21 days respectively) and panicle initiation (15.00, 16.00 and 16.31% in 7, 14 and 21 days respectively) stages. The TCP then rose during milk (15.73, 16.10 and 16.50% in 7, 14 and 21 days respectively) and soft dough (15.33, 15.80 and 16.39% in 7, 14 and 21 days respectively) stages to decline again during hard dough (12.20, 13.33 and 14.57% in 7, 14 and 21 days respectively) and physiological maturity stage (12.11, 13.43 and 14.73% in 7, 14 and 21 days respectively). Significant differences were shown in TCP in seedling, panicle initiation and physiological maturity stages where irrigation every 21 days yielded a high TCP% (16.40, 16.31 and 14.73% in seedling, panicle initiation and physiological maturity stages respectively).

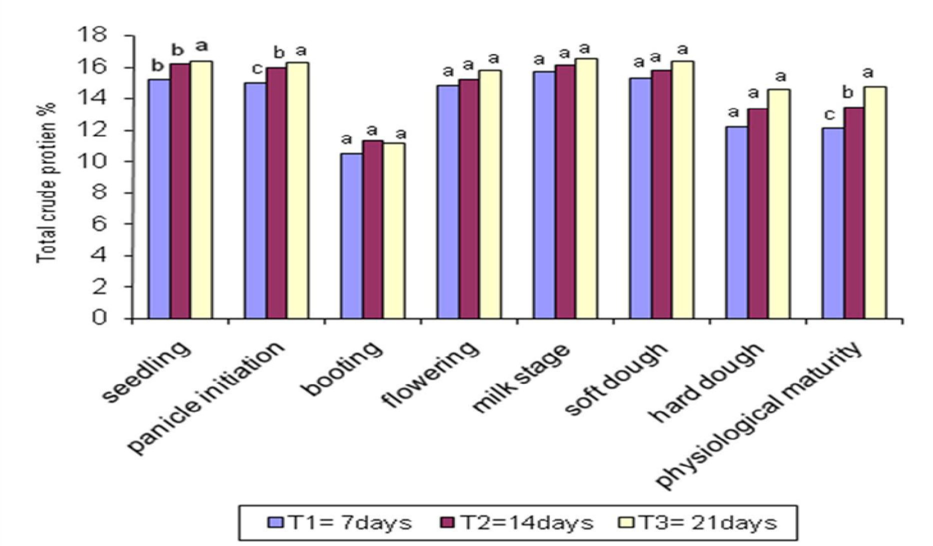


Fig.7. TCP% of Arfa Gadamk during different stages of growth (Season II)
*Comparisons were done within each growth stages individually at p ≤ 0.05 level of significant

**Total Crude Protein % of Tabbat Cultivar**

Figure 8 shows the variation in TCP% of Tabbat during different stages of growth in the second season. A clear reduction of TCP was observed at booting stage (10.31, 10.72 and 10.84% in 7, 14 and 21 days respectively) after the initial high levels in seedling (15.20, 16.00 and 16.00% in 7, 14 and 21 days respectively) and panicle initiation (15.07, 15.63 and 15.89% in 7, 14 and 21 days respectively) stages. The TCP then rose during flowering (14.61, 15.71 and 16.11% in 7, 14 and 21 days respectively), milk (14.80, 15.86 and 15.93% in 7, 14 and 21 days respectively) and soft dough (15.67, 16.37 and 16.71% in 7, 14 and 21 days respectively) stages to decline again during hard dough (13.87, 14.73 and 15.13% in 7, 14 and 21 days respectively) and physiological maturity (13.11, 14.58 and 15.23% in 7, 14 and 21 days respectively) stages. No significant differences were observed among different stages in response to watering treatments except in physiological maturity stage, where the long watering interval of 21 days gave the highest TCP (15.23%).

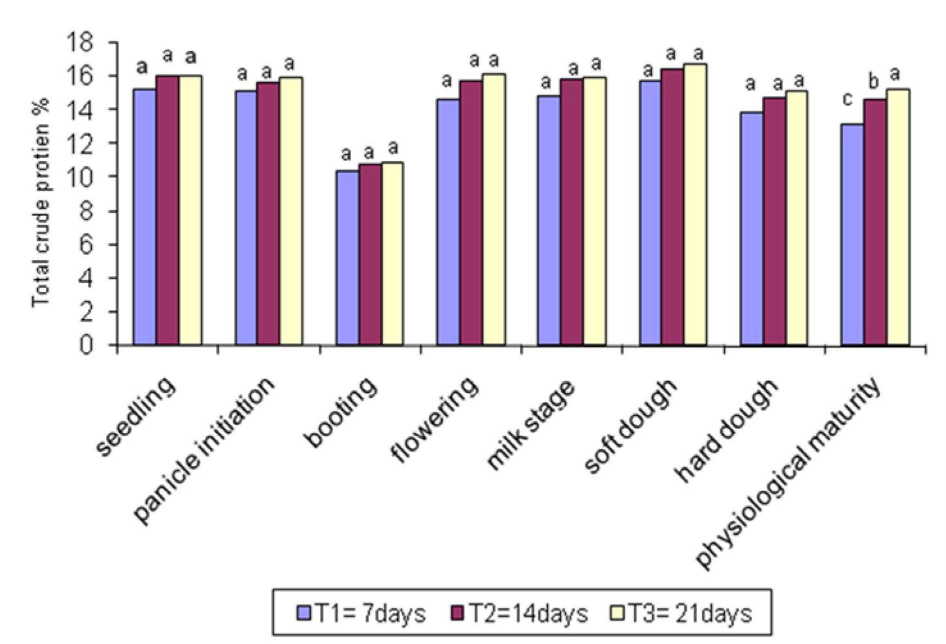


Fig. 8. TCP% of Tabbat during different stages of growth (Season II)
*Comparisons were done within each growth stages individually at p ≤ 0.05 level of significant

**Total Crude Protein % of Abu Sabien Cultivar**

Figure 9 reveals the variation in TCP of Abu Sabien during different stages of growth in the second season. A clear reduction of TCP% was observed in booting stage (10.03, 10.32 and 10.67% in 7, 14 and 21 days respectively) after the initial high levels in seedling (15.80, 16.70 and 16.40% in 7, 14 and 21 days respectively) and panicle initiation (15.11, 16.27 and 16.57% in 7, 14 and 21 days respectively) stages. It began to increase reaching its highest value in milk stage (15.44, 15.99 and 16.31% in 7, 14 and 21 days respectively). Water treatments significantly affected the TCP% in panicle initiation and soft dough stages, where the long watering interval of 21days produced the highest TCP (16.57% and 15.53% in panicle initiation and soft dough stages respectively).

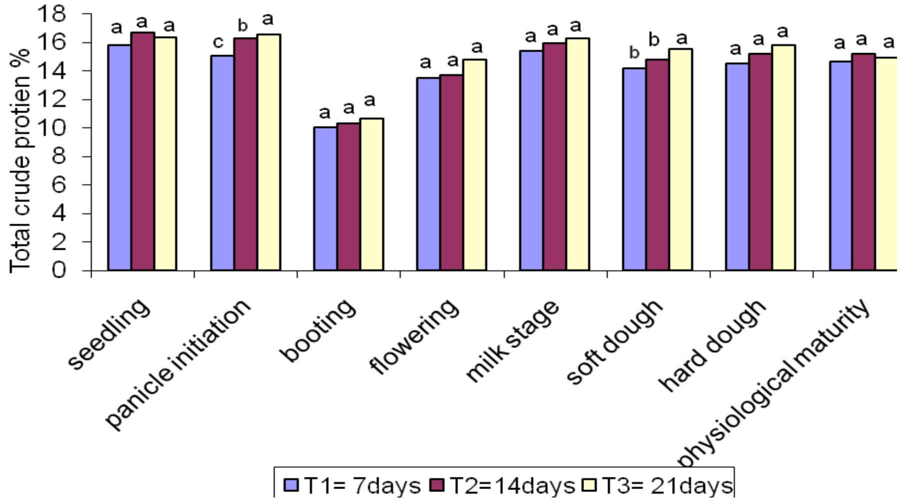


Fig. 9. TCP% of Abu Sabien during different stages of growth (Season II) *Comparisons were done within each growth stages individually at $p \leq 0.05$ level of significant

**Total Crude Protein % of White Mugud Cultivar**

Figure 10 shows the change in TCP% of White Mugud during different stages of growth in the second season. A clear reduction of TCP% was observed in booting stage (11.37, 10.99 and 11.61% in 7, 14 and 21 days respectively) after the initial high levels in seedling (16.71, 15.70 and 16.04% in 7, 14 and 21 days respectively) and panicle initiation (15.13, 15.60 and 16.07% in 7, 14 and 21 days respectively) stages. Thereafter, it began to rise toward flowering(15.02, 15.10 and 16.23% in 7, 14 and 21 days respectively) , milk (14.95, 15.68 and 16.59% in 7, 14 and 21 days respectively) and soft dough (15.58, 16.13 and 16.53% in 7, 14 and 21 days respectively) stages then it began to decrease. Significant differences were recorded in hard dough and physiological maturity stages, where the watering interval of 21 days yielded the high TCP (15.78% and 15.51% in hard dough and physiological maturity stages respectively.

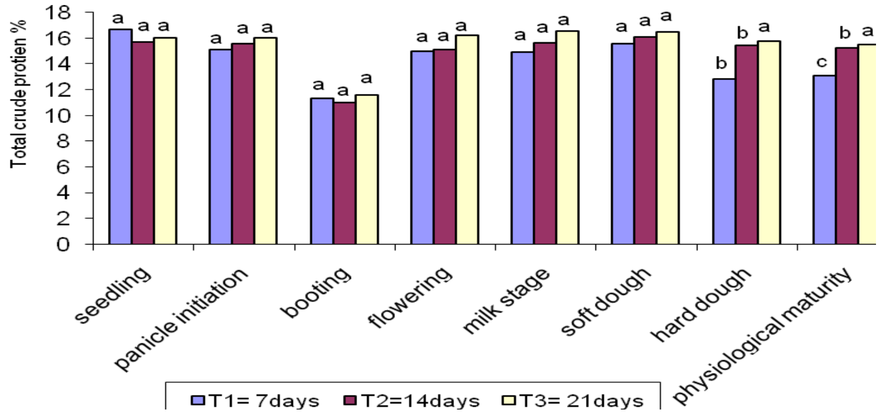


Fig. 10. TCP% of White Mugud during different stages of growth (Season II)
*Comparisons were done within each growth stages individually at $p \leq 0.05$ level of significant

## DISCUSSION

The total crude protein content varied with the stage of growth in all cultivars. The pattern of variation differed with the cultivar. Variation in protein content with the stage of growth is an established phenomenon in most plant species (Atta *et al.*, 2010; Anon, 2011; Olszewska, 2021). Protein content was usually found to be highest in the early stages of growth and declines with age (Anon, 2011and Olszewska, 2021) usually due to its translocation to the developing seeds. The findings of the present study confirm these earlier reports. In all five genotypes of this study, the highest protein content was found in the seedling stage. Annual plants are known to produce large amounts of proteins early in their lifecycle to meet the high demands for proteins required by the fast growth rates early in the season of growth (Anon., 2011). Thereafter protein content declined gradually and the absolute reduction was observed at booting stage or at flowering stage or at both stages of growth and then it slightly increased toward hard dough and physiological maturity stages. The gradual decrease in total protein of sorghum was probably due to increased- demand for proteins during booting and flowering (Anon, 2011).

There were increases in protein content in all growth stages in response to water stress. This was probably due to the production of dehydrins or stress proteins. These proteins are reported to confer stress tolerance to plants (Meenakshisundaram and Santhaguru, 2009; Hanin *et al.* 2011).

Dehydrin proteins accumulate along with other LEA proteins in response to a particular stress and have been proposed to play an important role in membrane protein stability and osmotic adjustment (Dure *et al*, 1989; Close, 1996; Liu *et al*. 2016) These observations suggest that dehydrins, as well as other LEA proteins, might play a role in the acquisition of desiccation tolerance in seeds (Dure *et al*, 1989, Dure, 1993a). Dehydrins have been most extensively studied in relation to drought stress (Halder *et al*, 2017; Riley *et al*, 2019; Tatenda *et al*. 2020). Proteins synthesized in response to drought stress are called dehydrins (dehydration induced) and belong to the group II late embryogenesis abundant (LEA) proteins (Also known as LEA D-11 family).

These stress proteins have chaperoned like functions .The tolerance conferred by stress proteins results in improved plant performance under stress conditions (Bao *et al*., 2017; Fan *et al*., 2017). A proposed role of dehydrin-like proteins in drought stress has been the protection of cells from dehydration stress. In arid and semi-arid environments, plants subjected to water stress are, usually, also subjected to heat stress. Stress proteins can confer tolerance against the two types of stress. Dehydrin-like proteins may also have a role similar to compatible solutes (such as proline, sucrose, and glycine betaine) in osmotic adjustment. Another possible role of stress proteins is to bind with the ions accumulated (ion sequestering) under drought stress and to control solute concentration in the cytoplasm (Yang *et al*., 2021).

In the absence of stress several stress proteins were found to assume important cellular functions such as aiding in folding, protein translocation across membranes and the control of degradation (Hanin *et* *al.* 2011). Stress proteins have protective functions during stress. They prevent protein denaturation and unfolding and are involved in photosynthetic thermo tolerance by protecting photo system II and the oxygen evolving complex at high temperatures (Liu *et al.* 2016).

## CONCLUSIONS

It could be concluded from this study that drought stress caused significant genotypic differences in TCP% in season two at seedling stage, panicle initiation stage, flowering stage, milk stage, soft dough stage, while at hard dough and physiological maturity stages drought stress affected TCP% in both seasons. Watering treatments had no significant effect on TCP% in season one at seedling stage, panicle initiation, booting, flowering stage, milk stage, soft dough (leaves and seeds) and hard dough seed. A high TCP% was found in the initial stages of growth in all genotypes followed by a clear reduction at booting or flowering stages, and a subsequent rise towards physiological maturity. Water stress positively affected TCP%. Most genotypes under the long watering intervals of 21 days yielded a high TCP%.

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## تقييم البروتين الكلي خلال نمو و تطور بعض سلالات الذرة الرفيعة النامية تحت الاجهاد المائي*

هدى عبده حسن[1] والطيب الحاج على أحمد[2] والهادي على إبراهيم[3]

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**مستخلص البحث**: درست التغييرات في النسبة المئوية للبروتين الكلي خلال نمو وتطور بعض اصناف الذرة الرفيعة تحت ظروف الاجهاد المائي في تجربة حقلية لموسمين (2018/2017) و (2019/2018) بالمزرعة التجريبية بكلية الزراعة، شمبات، جامعة الخرطوم. استعمل تصميم القطع المنشقة بثلاثة مكررات وكانت معاملات الري كل 7 (T1)، 14 (T2) و 21 (T3) يوم في الاحواض الرئيسية واصناف الذرة ود أحمد، أرفع قدمك، طابت، أبو سبعين و المقد الأبيض في الاحواض الصغيرة. جمعت الاوراق لدراسة النسبة المئوية للبروتين الكلي خلال مراحل نمو الذرة (طور البادرة، الطور الابتدائي للعنقود الزهري، الطورالتمهيدي للازهار، طور التزهير، طور اللبنة، طور العجينة اللينة، طورالعجينة القاسية و طور النضج الفسيولوجي). دلت النتائج ان هنالك فروقات معنوية للنسبة المئوية للبروتين الكلي بين الاصناف وسجلت أعلى قيمة (2, 15%) لود احمد في الشاهد ، اقل قيمة مسجلة (8,4%) للمقد الابيض في الشاهد على عكس الاصناف الاربعة الاخرى،انخفض البروتين الكلي لابوسبعين من 8, 13 %في الشاهد الى 10,6 % تحت ظروف الاجهاد .النسبة المئوية للبروتين الكلي تنخفض تدريجياً ناحية الطورالتمهيدي للازهار و طور التزهيرفي معظم السلالات. معظم السلالات في فترة الري الطويل (21 يوم) انتجت اعلي نسبة مئوية للبروتين الكلي.

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* جزء من رسالة دكتوراه من جامعة الخرطوم للمؤلف الاول

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