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Relationship Between Kernel Ash Content, Water Use Efficiency and Yield in Durum Wheat under Water Deficit Induced at Different Growth Stages

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Abstract: Drought is the main factor affecting crop grain yield in many semi-arid and arid regions of the world. Increasing grain yield under drought and crop water use efficiency are essential for enhancing crop production in water-limited environments. The aim of this study was to determine the relationship between kernel ash, water use efficiency and grain yield of durum wheat genotypes under water deficit conditions induced at three growth stages. The result revealed that water deficit significantly affected grain yield, biomass yield, harvest index, kernel weight, kernel number per spike, kernel ash content and water use efficiency. Kernel ash content was negatively and significantly correlated with grain yield and WUE under the prolonged water deficit induced at tillering and anthesis stages and such relationship did not appear under moderate water deficit induced at grain-filling stage and well-watered control. The relationship between kernel ash and 100-kernel weight was consistently negative and significant in all water regimes. On other hand, kernel ash content was positively correlated with drought susceptibility index under severe water deficit showing that susceptible genotypes during grain-filling accumulated more minerals in their kernels. The study result suggested that ash content in kernels is a useful physiological tool for assessing yield performance and water use efficiency in durum wheat under severe water deficit conditions.

Key words: Durum wheat · Water deficit · Kernel ash content · WUE

INTRODUCTION

Durum wheat production in many arid and semi-arid regions of the world is constrained by water stress. Water use efficiency (the ratio of dry matter production to water consumed) is considered as a trait contributing to successful growth and production in water-limited environments [1]. However, its direct measurement is tiresome, expensive and time consuming, especially in field trials, limiting its use in breeding programs. Carbon isotope discrimination (?) in plant tissues is considered to be an integrated measurement of water use efficiency [2]. During photosynthesis, $^{13}CO_2$ is subjected to discrimination and this leads to depletion of ^{13}C in plant material. Carbon isotope discrimination was found to be positively related with grain yield for many C_3 cereals including durum wheat [3-6] grown under different water regimes. Since ? is a highly heritable trait [7] and found to be associated with molecular markers, it has been proposed to be used in breeding programs instead of molecular markers [8]. Stable isotopes have proved a valuable phenotyping tool in predicting water use efficiency in drought-prone areas, however, the cost and technical skill in isotope analysis limits its large application in breeding programs particular in developing country [3, 9]. As a result, much work has been focused on finding cheap and easily determined putative surrogate of ?. The accumulation of minerals (measured as ash content) in both vegetative tissue [10 -12] and kernels [1, 3, 4, 13] has been proposed as inexpensive and simple way to predict drought adaptation and yield in C_3 cereals.

The mechanism of ash accumulation in vegetative tissue appears to be explained through the passive transport of minerals via xylem driven transpiration [10, 11, 14]. Conversely, minerals accumulation in kernels takes place via phloem [15]. The vegetative ash could provide information about photosynthetic and transpirative gas-exchange activity [3, 14] while kernel ash could provide information on the integrated performance of photosynthetic and translocation process during grain

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filling [3, 4, 13, 14]. The ash concentration in mature kernels could also indicate the importance of re-translocation process during grain filling [1] such that genotypes more affected by drought during grain filling have higher grain ash content [3]. The heritability of ash content greater than grain yield in durum wheat [3], indicating that the trait is under genetic control and likely to be altered through breeding.

The objective of the present study was to identify traits associated with grain yield under water deficit induced at three growth stages. The potential value of grain ash (G_am_a) as indirect selection criteria for grain yield and water use efficiency was investigated.

MATERIALS AND METHODS

Planting Materials: The study was conducted in a lathhouse at Sinana Agricultural Research Center (SARC) during the 2006/07 main season. It is located at 7° 7'N latitude, 40^0 10' E longitude and 2400 m.a.s.l altitude in Bale Zone of Oromia Region, Ethiopia. To embrace the variability existing among the Ethiopian durum wheat genotypes, three landrace [B5-5B, S-17B and WA-13], thirteen commercial cultivars [Asassa, Bekelcha, Boohai, Egersa, Foka, Gerardo, Ilani, Kilinto, Obsa, Oda, Quamy, Tob-66 and Yeror] and two advanced lines [CDSS93Y107 and CD94523] from the breeding program were used. The examined genotypes are different in genetic background, origin and several characteristics.

Experimental Design and Producers: Plants were grown in 21 cm diameter and 18 cm length plastic pots filled with a textural class of clay (49.7% clay, 27.3% silt and 23% sand). Each pot was filled with 4 kg uniformly air-dried soil (17.1% moisture). The field capacity and permanent wilting point of the soil were 47.8% and 11.5%, respectively. Pots were arranged in Randomized Complete Block Design (RCBD) in factorial combination of the eighteen genotypes and four water regimes with three replications. A total of 216 pots, 12 pots were assigned to each genotype. 2g N and 2 g P_2O_5 fertilizers were applied to each pot during planting and additional 0.5 g N was applied at the first tillering. Planting was done on August 10, 2006. Eight seeds were sown per pot and the seedlings were thinned to four at two leaf growth stages. Five hundred ml of water was added to each pot every other day for a period of a month until the plants reach four leaf growth stages.

Water Deficit Treatments: Following the Zadock's scale [16], plants were subjected to water deficit at different growth stages: deficit continuously from tillering to physiological maturity (hereafter called M1), deficit from anthesis to physiological maturity (M2) and deficit from grain-filling stage to physiological maturity (M3) and well-watered control (C) treatments. The water levels were maintained in the range of 35-50% field capacity in the deficit treatments while above 75% in the control treatment. These water stress conditions are designed to simulate the environments that experience very low water supply after crop establishment in different parts of the country. During the stress period, plants were left without water for 12 days by withholding irrigation until early morning wilting is observed. Then pots were weighted and irrigated until the weight of every pot became equal to the weight of the predetermined water level. The amount of water depleted from pots was obtained by weighing pots every two to three days and the loss in weight was restored by watering pots with the amount of water equal to the loss in weight.

Measurements

Yield and Yield Component: Data were collected for number of kernels per spike, 100 kernel weight, air-dried aboveground biomass and grain yield per plant. Harvest index was determined as the proportion of grain yield to the overall aboveground biomass per plant.

Crop Water Use Efficiency: water use efficiency on biomass basis (WUE_B) and water-use efficiency on grain basis (WUE_G) were determined as ratio of biomass and grain yield to seasonal plant water use, respectively. The seasonal water use was obtained by summing up the difference in soil water between measurements just before and after irrigation of each pot.

Kernel Ash Content (M_ag_a),: Which is expressed in dry weight basis (%), was determined from the kernel after complete combustion of the grain powder. Samples from each replicate were bulked together and grounded to a fine powder and oven-dried for 48 h at 72°C. Each sample was divided into two replicates. Approximately 3 g of grain powder was incinerated at 575 °C for 16 h (until light gray ash was obtained) in a muffle electric furnace and then the weight of the residue was recorded.

Drought Indices: Drought susceptibility index (S) was calculated from genotype means by using the generalized formula of Fischer and Maurer [17] and geometric mean productivity (GMP) for each genotype was also calculated following the procedure given by Fernandez [18].

Statistical Data Analysis: The data were subject variance analysis using SAS GLM procedure release 8.02 (SAS Institute inc. 2001). Means comparisons were carried out to estimate the differences between water deficit treatments and genotypes using Duncan's Multiple Range Test values. Linear correlation analyses were used to determine the association between grain yield and physiological traits using SPSS (SPSS 12.0.1).

RESULTS AND DISCUSION

Grain yield varied significantly among water regimes between 0.79 (at tillering stage) and 2.81 gm plant^{-1} (control) (Table 2). There are also highly significant differences in biomass yield, harvest index, hundred kernel weight and number of kernels per spike, seasonal water use and water use efficiency among water regimes. Significant genotypic variation ($P < 0.01$) were also detected in all agronomic traits including kernel ash and water use efficiency (Table 1).

Blum [19] argued that genotypic variation in WUE under limited water regimes is affected more by variation in the denominator (WU) rather than by variation in the nominator (biomass). Analysis of covariance, using seasonal water use per plant as covariate, was performed in order to test if seasonal water use could be the origin of WUE_G and WUE_B variation among genotypes. Indeed, significant genotypic variability was found after correction for seasonal water use. Therefore, it appears that the genotypic variability for WUE_G and WUE_B was not only attributable to differences in seasonal water use but also attributable to differences in grain yield and aboveground biomass productions. It is well established that high grain yield under water-limited condition is the function of the amount of water used for growth, WUE and HI [20]. Moreover, plant biomass production depends on the amount of water used for growth as well as WUE. Therefore, WUE could be of a particular interest in situations where growth is affected because of water availability [21, 22]. The high variability among Ethiopian durum wheat genotypes for $WUE_{G\ and}$ WUE_B found in the present study as well as reported by Solomon and Labuschagne [23] suggesting the greater opportunity for selecting superior genotypes adapted to water-limited environments.

Table 1: Analysis of variance showing the mean squares of yield and other yield attributes of 18 durum wheat genotypes as influenced by water deficit treatments at three growth stages

	Mean squares				
Variables	Moisture Deficit (M) (*df* = 3)	Genotypes (G) (*df* =17)	M x G (*df* =51)	Error (*df* =136)	*CV (%)*
Grain yield	35.9***	0.87***	0.43***	0.11	17.4
Biomass yield	212.3***	1.93***	1.34***	0.52	15.5
Harvest index	0.57***	0.0057***	0.0062***	0.0014	9.2
100 kernels weight	9.82***	2.03***	0.44***	0.124	7.8
Kernels number per spike	4823.3***	205.9***	49.9***	14.9	12.7
Total seasonal water use	24.8***	0.11***	0.16***	0.037	8.0
WUE_G	1.37***	0.14***	0.038***	0.016	16.7
WUE_B	7.71***	0.30***	0.15**	0.15	15.5
M_aG_a	8.20***	0.198***	0.077**	0.012	8.7

NS, ** and ***= not significant, significantly different at 1 % and 0.1% level of probability, respectively.

Table 2: Mean values of grain yield (gm plant^{-1}), biomass yield (gm plant^{-1}), HI, 100-kernel weight, kernel number per spike, seasonal water use (kg plant^{-1}), WUE (gm kg^{-1})and kernel ash content (M_aG_a; % in dry weight basis) for each water regimes involving 18 durum wheat genotypes

Water deficit	GY	Biomass	HI	KWT	No. kernel spike^{-1}	WU	WUE_G	WUE_B	M_aG_a
[1]M1	0.79	2.10	0.36	4.48	16.6	1.52	0.53	1.39	1.99
M2	1.77	4.19	0.41	3.92	32.3	2.28	0.78	1.95	1.06
M3	2.33	5.32	0.42	4.57	36.7	2.61	0.79	2.12	0.94
C	2.81	6.60	0.43	5.02	37.6	3.15	0.85	2.11	1.17
Mean	1.93	4.55	0.41	4.50	30.8	2.39	0.74	1.89	1.29
LSD (P<0.05)	0.11	0.24	0.01	0.12	1.29	0.06	0.04	0.25	0.05
CV (5%)	17.4	15.5	9.2	7.8	12.7	8.01	16.7	15.5	8.7

[a]M1= water deficit treatment induced from tillering to physiological maturity; M2= water deficit treatment induced from anthesis to physiological maturity; M3= water deficit treatment induced from grain-filling to physiological maturity; C= well-watered treatment throughout the entire growth period (control).

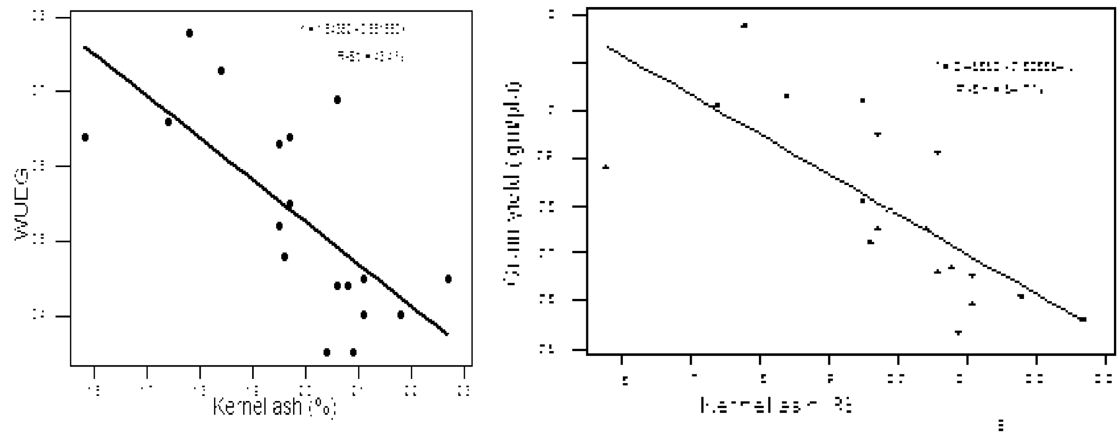

Fig. 1: Relationship between kernel ash and grain yield per plant and WUE_G under water deficit induced at tillering to crop physiological maturity

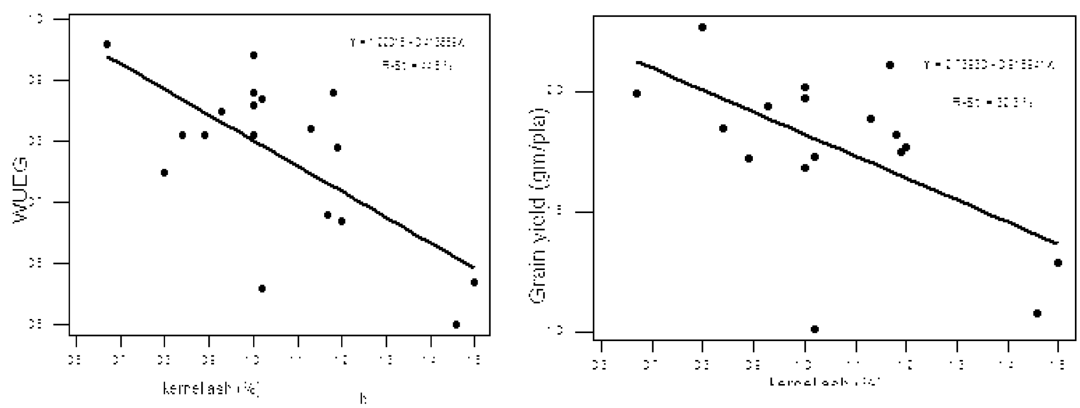

Fig. 2: Relationship between kernel ash and grain yield per plant and WUE_G under water deficit induced at anthesis to crop physiological maturity

The mean kernel ash concentration values differed significantly under water deficit treatments (Table 2). Mean ash accumulated (on dry mass basis) in the kernels under water stress induced at tillering stage was higher by 70.1% as compared to the well-watered treatment. An increase in kernel ash content under water stressed condition was also reported by Ozturk and Aydin [24]. However, the mean values of the kernel ash content at anthesis and and grain-filling stages were lower than the values in the well-watered control treatment. Lower hundred kernel weight was also observed under water deficit induced at these stages (Table 2). This might be as a result of the observed leaf senescence and marked reduction of grain-filling period and grain filling rate under water deficit conditions. Mean kernel weight was associated to the length of grain filling period and grain filling rates [25]. The reduction of the time for photo-assimilate translocation to the grain which might also reduces both the sink size and mineral accumulation in the stressed treatments. The mineral accumulation in kernels primarily depends on re-mobilization from leaves and stems and on the minerals removed from the vegetative parts of the plant after onset of senescence [26]. Thus, re-mobilization of minerals from vegetative tissue usually leads to an increase in grain ash content [1, 10].

Kernel ash content was negatively significantly correlated with grain yield under the stress induced at tillering and anthesis stages (Fig 1 and 2). Such relationship, however, did not exist in the moderate stress induced at grain-filling stage and control treatment (Fig 3). This is an agreement with the previous reports on bread and durum wheat [27, 28] and barley [4] in which only the m_aG_a in the rainfed environment showed a negative relationship with grain yield. On the other hand, negative relationship between kernel ash content and grain yield both under stressed and irrigated environments was

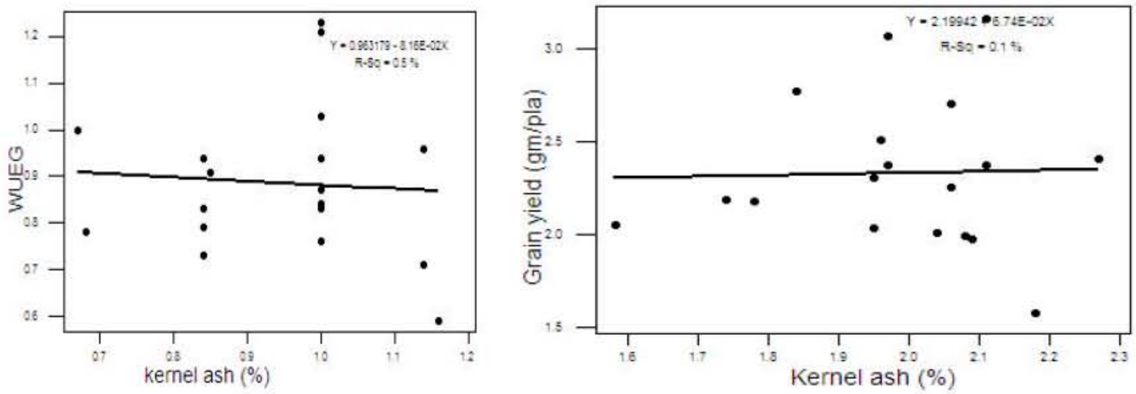

Fig. 3: Relationship between kernel ash and grain yield per plant and WUE_G under water deficit induced at grain filling stage to crop physiological maturity

Table 3: Correlation coefficients of the relationship between kernel ash content and grain yield and other yield components of durum wheat genotypesgrownunder water deficit treatment induced at three growth stages

Water Deficit	[a]GY	Biomass	HIK	WT	KSPK	WUE_G	WUE
$_B$[b]M1	-0.74***	-0.72**	-0.53**	-0.59**	-0.68**	-0.70**	-0.63**
M2	-0.57**	-0.44	-0.30	-0.52**	-0.11	-0.67**	-0.39
M3	-0.01	-0.28	-0.02	-0.60**	-0.46*	-0.07	-0.16
C	-0.35	-0.32	-0.10	-0.49*	-0.33	-0.11	-0.23

*P < 0.05; ** P < 0.01, *** P < 0.001, [a] GY= Grain yield per plant (g/plant), Biomass = Aboveground biomass per plant (g/plant), HI = Harvest index, KWT= Hundred kernel weight (gm), KSPK = Number of kernels per spike, WUE_G= Grain yield water use efficiency and WUE_B = aboveground dry matter water use efficiency.

reported by many authors [1, 3, 5] although the magnitude of the correlation was higher under more severe stress conditions while Monneveux *et al.* [29] did not report such a relationship under irrigation in wheat. These results suggest that the relationship between grain yield and kernel ash content of wheat may depend more on stress intensity than on stress modality.

Negative and significant correlation was also noted between kernel ash content and both biomass yield and harvest index under water deficit induced at tillering stage. The relationship between kernel ash content and biomass yield and between kernel ash content and harvest index were negative but insignificant at anthesis, grain-filling stages and well-watered condition (Table 3). The negative association between kernel ash content and harvest index agrees with previous report which indicated that genotypes that had lower kernel ash content are more efficient in dry matter partitioning to the grain and therefore can produce higher yield in severe water deficit conditions [5]. The relationship between kernel ash and 100-kernel weight was consistently negative and significant in all the water regime treatments. A strong negative relationship between kernel ash and kernel weight has been reported previously by Araus *et al.* [3] suggesting that mineral accumulation in kernels appeared to be less affected than photoassimilates by environmental constraints during grain-filling. Negative and significant correlation was also found between kernel ash content and number of kernels per spike under water deficit induced at tillering and grain-filling stages. Similarly, the kernel ash content was significantly negatively correlated with the number of kernels per spikelet at both in water deficit induced at tillering stage and well-watered condition treatments (Table 3). Grain yield showed a positive significant correlation with WUE_G and biomass yield under all water regimes. Solomon and Labuschagne [23] also reported positive correlation between WUE and grain yield. Therefore, increased aboveground biomass per plant and increased water use efficiency are inductive of high grain yield potential under water stress conditions.

The relationship between kernel ash and both WUE_G and WUE_B were found to be negative and significant under water deficit induced at tillering (Table 3). Similarly, WUE_G was negatively significantly correlated with kernel ash content under water deficit induced at anthesis. The presence of negative association between kernel ash content and WUE agrees with many investigators who

Table 4: Correlation coefficients of the relationship between kernel ash content (m_aG_a; % in dry weight basis) and two drought indices in durum wheat genotypes grown under water deficit treatment induced at three growth stages

Water deficit	S	GMP
M1	0.68**	-0.66**
M2	0.49*	-0.49*
M3	0.12	-0.03

* and ** significant at $P < 0.05$ and 0.01, respectively.

reported water use efficiency on biomass basis [30] and/or on the basis of carbon isotope discrimination (?) analysis [1, 3, 5 and 6]. The authors reported that genotypes with higher transpiration per unit of dry matter produced (low TE, high ?) had higher ash content in leaf dry matter and lower kernel ash (m_aG_a). Misra *et al* [30] also suggested the usefulness of kernel ash as indicator of the quantity of water transpired during the growth cycle and WUE across environments.

Kernel ash content was positively and significantly correlated with drought susceptibility index (S) under severe water deficit induced at tillereing and anthesis stages (Table 4), indicating that kernel ash was increased in those genotypes susceptible to drought stress during tillering and anthesis periods. Similarly, Araus *et al* [3] and Merah *et al* [5] reported that genotypes more affected by drought during grain filling have high grain ash content. However, significant and negative relationship was observed between GMP and kernel ash at those stages (Table 4).

CONCULUSION

Results obtained in this experiment indicated that water deficit induced at different growth stages significantly affected all the yield components including kernel ash and water use efficiency. Kernel ash content was negatively and significantly correlated with grain yield and WUE under the prolonged water deficit that induced at tillering and anthesis stages and such relationship did not exist under moderate water deficit induced at grain-filling stage and well-watered control indicating that kernel ash content of wheat may depend more on stress intensity than on stress modality. The relationship between kernel ash and 100-kernel weight was consistently negative and significant in all the water regime treatments. Kernel ash content was positively correlated with drought susceptibility index under severe water deficit showing that susceptible genotypes during grain-filling stage accumulated more minerals in the kernels. The result highlights the kernel ash content as a useful indicator of grain yield and WUE_G in durum wheat and might provide as indirect selection criterion for grain yield and water use efficiency under severe water deficit conditions.

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