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EVALUATION OF BIO-PHYSIOLOGICAL AND YIELD RESPONSES OF STAY GREEN QTL INTROGRESSION SORGHUM LINES TO POST-FLOWERING DROUGHT STRESS

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ABSTRACT

Drought is the most drastic abiotic stress that limits crop production in sub-Saharan Africa. Sorghum (*Sorghum bicolor* (L.) Moench) is among the most climate resilient cereals of the future due to its tolerance to drought and heat. Thus, identification of tolerant genotypes and plant characteristics that contribute to drought adaptation is timely. The objective of this study was to evaluate the response of 12 genotypes, including seven Stay-green (*Stg*) QTL introgression sorghum lines and their two donor; and three recurrent parents, to induced post-flowering drought stress. The recurrent parents were varieties released for drought prone lowlands of the country, and the donor parents were lines known to have stay-green properties. The study was conducted at Werer, in the Great Rift-valley of Ethiopia, during the off-season of 2014 under well-watered and water limited conditions. Significant differences ($P < 0.05$) were observed among genotypes under water deficit regimes, for all measured physiological, biochemical (proline content), yield and yield related traits. Three *Stg* QTL introgression lines showed better tolerance to drought than their recurrent parents, as reflected by yield and maintenance of green leaf area. Three *Stg* QTL introgression lines, Teshale/E36-1, Gambella/B35-5 and Meko/B35-12, gave the highest grain yield under post-flowering drought stress, and B35 and Teshale/B35 gave the poorest yield under similar conditions. Therefore, these improved lines bear potential for use as alternatives in breeding programmes and for possible release in parts of the country where post-flowering drought stress is frequent. Proline content, weight of hundred seeds (HSW) and CO_2 assimilation are effective screening tools for *Stg* property due to their high correlation with other parameters.

Key Words: Post-flowering, proline, *Sorghum bicolor*

RÉSUMÉ

La sécheresse est le stress abiotique le plus drastique qui limite la production agricole en Afrique Sub-saharienne. Le sorgho (*Sorghum bicolor* (L.) Moench) est parmi les céréales les plus résilientes au climat pour le futur dû à sa tolérance à la sécheresse et à la chaleur. Donc, l'identification des génotypes tolérants et les caractéristiques des plants qui contribuent à l'adaptation à la sécheresse est opportune. L'objectif de cette étude était d'évaluer la réponse de 12 génotypes, comportant sept lignées de sorgho conférant l'introgression des QTL des attributs verts (*Stg*), leurs deux donneurs ; et les trois parents récurrents, au stress de la sécheresse post floraison induite. Les parents récurrents ont été les variétés libérées sensibles à la sécheresse dans les plaines du pays, et les donneurs ont été les lignées connues pour leurs propriétés relatives aux attributs verts. L'étude a été conduite à

Werer, dans la grande vallée du Rift d’Ethiopie, pendant la contre saison de 2014 dans des conditions d’irrigation abondante et d’irrigation limitée. De différences significatives ($P < 0.05$) ont été observées entre les génotypes sous des régimes de déficit hydriques, pour tous les traits physiologiques, biochimiques (teneur en proline), le rendement et les composantes de rendement. Trois lignées d’introgression de *Stg* QTL ont montré de meilleure tolérance à la sécheresse que leurs parents récurrents, comme reflété par le rendement et la maintenance de la surface verte de la feuille. Trois lignées d’introgression de *Stg* QTL, Teshale/E36-1, Gambella/B35-5 et Meko/B35-12, ont donné le plus haut rendement sous le stress de sécheresse de post floraison, et B35 et Teshale/B35 ont donné le plus faible rendement dans des conditions similaires. En conséquence, ces lignées améliorées détiennent tout le potentiel pour leur usage comme alternatives dans des programmes d’amélioration génétiques et pour une libération possible dans les régions du pays où le stress de la sécheresse post floraison est fréquent. La teneur en proline, le poids de 100 graines (HSW) et l’assimilation du CO_2 sont des outils effectifs de criblage pour la propriété de *Stg* due à leur forte corrélation avec les autres paramètres.

Mots Clés: Post-floraison, proline, *Sorghum bicolor*

INTRODUCTION

Drought is one of the primary causes of household food insecurity in sub-Sahara Africa, as the food production trend correlates with the rainfall pattern (Bekele, 2001). Drought stress occurs anytime during the life cycle of the crop (Adugna and Tirfesa, 2014). However, major crop yield decline due to moisture deficit during flowering and grain filling stages (Ali *et al.*, 2011); by reducing the number and weight of seeds (ICRISAT, 2013).

Sorghum (*Sorghum bicolor* (L.) Moench) can adapt better to adverse growing conditions such as drought, than other cereals such as maize (*Zea mays* L.) (Dahlberg *et al.*, 2011). It can produce grain in areas too dry for maize; such as areas having less than 250 mm average annual rainfall (Chamberlin and Schmidt, 2011). Sorghum genotypes differ in their ability to avoid leaf senescence and maintain green leaf area until maturity (Adugna and Tirfessa, 2014). Leaf senescence is symptomised by visual leaf symptoms such as loss of chlorophyll pigments (yellowing), wilting and eventual abscission (He *et al.*, 2005).

Stay green (*Stg*) is a property of plants to maintain prolonged green leaf area and stalk under post-flowering drought stress (Rosenow *et al.*, 1983). Sorghum genotypes that possess *Stg* trait are also reported to have reduced lodging and resistance to stalk rots (Borrell *et*

al., 2000). It is used as a strategy to reduce the effect of drought at grain filling, as drought stress during this stage in sorghum usually results in rapid premature plant senescence, which may in turn reduce grain yield. Therefore, *Stg* is considered an important genetic trait associated with drought tolerance and has been mapped to a number of key chromosomal regions (QTLs) in sorghum (Borrell *et al.*, 2014).

The International Crops Research Institute for the Semi-arid Tropics (ICRISAT) and the Ethiopian National Sorghum Improvement Programme at Melkassa Agricultural Research Center (MARC) carried out collaborative marker assisted backcrossing (MAB) (Adugna and Tirfessa, 2014), to improve the terminal drought tolerance of the locally adapted high yielding varieties. As a result, a fairly large number of *Stg* QTL introgression sorghum lines were developed. Three separate experiments were organised to test the various QTL introgression lines under post-flowering drought stress. Adugna and Tirfessa (2014) evaluated one set of the lines for morphological, yield and yield related characters. In addition to these characters, Sintayehu *et al.* (2018) evaluated another set of introgression lines (selections) for physiological and root characters in addition to yield and yield related traits. Both studies found that many of the introgression lines were better than their susceptible recurrent parents for key traits. However, we believed that

inclusion of proline content in the study might reveal better insights about the response of *Stg* introgressed lines under drought stress. This study was done concurrently with the latter study and designed to include proline. The objective of the present study was to determine the extent of drought stress tolerance of the *Stg* introgressed lines through biophysiological and yield related traits.

MATERIALS AND METHODS

Study area. Stay-green QTL introgression was done at Melkassa Agricultural Research Center from 2006 to 2008. The field experiment was conducted during the off-season of February to June 2014 at the experimental field of Werer Agricultural Research Center (40°11'E, 09°22'N, altitude 750 m asl), located in the Afar Regional State, 280 Km east of the capital, Addis Ababa in Ethiopia. The site soil was black alluvial, with a pH and electrical conductivity of 6.9 and 0.78 dS/m, respectively. Its annual rainfall ranged from 200 to 500 mm, with mean minimum temperatures of 15.2 °C in December, 23 °C in June and the mean maximum temperatures are 32.5 °C in December and 38 °C in June. Weather data during the experimental periods were collected from Ethiopian Meteorological Agency (EMA) and showed that there was small rainfall during the period of the experiment (EMA, 2014).

Plant material. Seven *Stg* QTL introgression sorghum lines, which were derived through marker assisted backcrossing (Gambella 1107/B35-1, Gambella 1107/B35-5, Gambella 1107/E36-1, Teshale/E36-1, Teshale/B35, Meko/B35-8 and Meko/B35-12); two known *Stg* QTL donor parents (B35 and E36-1); and three senescent recurrent parents (Gambella 1107, Teshale and Meko), constituted the experimental materials. Seeds of all of the 12 varieties and lines (hereafter referred to as genotypes) were obtained from the Ethiopian Sorghum Research Programme at Melkassa

Agricultural Research Center. The recurrent parents were high yielding varieties, released for drought prone environments, but their yield performance had been repeatedly affected by post-flowering drought stress (Adugna and Tirfessa, 2014). Moreover, they were known to have good grain quality for the preparation of local food stuffs (Adugna, 2007).

The variety Gambella 1107 was an Ethiopian landrace selection, originally collected from Gambella region; while Meko (M36121) and Teshale (3443-2-OP) were exotic sorghum varieties introduced from ICRISAT and released in Ethiopia in 1997 and 2002, respectively (Adugna, 2007). The *Stg* donor parent, B35, was a durra sorghum, a derivative of a landrace germplasm accession from Ethiopia (Reddy *et al.*, 2009). It was the best characterised source of *Stg* for post-flowering drought tolerance in sorghum. E36-1 was also a known source of *Stg* trait being used in breeding programmes and was of Ethiopian origin (Reddy *et al.*, 2009).

Experimental procedures. Treatments included 12 sorghum genotypes (seven QTL introgression lines, two *Stg* donor lines and three senescent recurrent parents) and two irrigation regimes (well-watered and water limited). The experiment was laid out in split-plot design with three replications. The two irrigation regimes were assigned as main plot factors, whereas the 12 sorghum genotypes were the sub-plot treatment factors. The plots in the water limited treatment were irrigated until flowering and, thereafter, irrigation was withheld. Those in well-watered treatment continued irrigation until physiological maturity. Irrigation was done using fresh water pumped directly from River Awash every seven days by flooding in the furrows, until the furrows were full. Each subplot consisted of three rows of 3.5 m length and 0.80 m inter-row spacing. The genotypes were randomised in each main plot.

Seeds of the experimental genotypes were sown by hand drilling in rows. They received

the first irrigation on February 3, 2014. Two weeks after emergence, the plants were thinned to one plant per hill to maintain the recommended spacing of 15 cm.

Fertilisers were applied as per the local recommendations (Reddy and Georgis, 1993). Accordingly, 36.23 g P₂O₅ and 14.18 g N, which is equivalent to 100 kg per hectare of DAP (46%P₂O₅, 18%N) was applied per plot in seed furrows during sowing; and 36.23 g of N per plot, which is equivalent to 100 kg per hectare of urea was applied as top dressing when the plants reached at knee height stage.

The experimental field was kept free from weeds throughout the study period by hand weeding. Moreover, Karate 5% EC, was sprayed at the rate of 320 mm per hectare as needed to control stem borers and shoot flies.

Data collection and analysis. Data were recorded on five plants, which were randomly tagged in each plot in three ways. Leaf relative water content (RWC) and chlorophyll content were recorded twice, on the 30th day after the date of 50% flowering and on the 15th day after the date of first measurement on the tagged plants. Leaf *Stg* rating was recorded at physiological maturity. Yield and yield related parameters were recorded after harvesting.

Leaf senescence rating at physiological maturity, which is reportedly an excellent indicator of *Stg* (Sanchez *et al.*, 2002), was recorded at this stage. The rating was done using 1 to 5 scales by visually observing the leaf area and determining whether it was senesced, following the procedure of Rosenow (1993). Completely green leaves without any leaf area death were rated 1 and leaves with leaf area death from 1-25%, rated 2, 26-50%, rated 3, 51-75%, rated 4, 76 to 100 % were rated 5 (Rosenow, 1993).

Gas exchange measurements (CO₂ assimilation rate and transpiration rate) were measured simultaneously using Lc pro+ portable photosynthesis system (ADC Bioscientific Ltd, Hoddesdon, England). Lc pro+ portable photosynthesis system measures

gas exchange in open system in which fresh air is passed through the plant leaf chamber on a continuous manner. When CO₂ is taken up by leaves in the process of photosynthesis, it induces more atmospheric CO₂ to diffuse through stomata, while water vapour can easily diffuse out by the process of transpiration.

Measurements were taken early in the morning on a fully expanded, deep green, and healthy leaves, which were fully exposed to sun light to capture their full function. The average of the five sampled plants was recorded as plot value. Water use efficiency (WUE) was calculated by taking the ratio of CO₂ assimilation rate to water lost due to transpiration (Barrs, 1968).

Chlorophyll content index reading was also taken using a hand held Chlorophyll Content Meter/ CCM-200 plus (Apogee Instruments, Inc. Logan, USA). The ratio of absorption of the light emitted from the instrument at two wave lengths (931 nm 653 nm⁻¹) is the basis for the measurement of chlorophyll content index (www.apogeeinstruments.com). As the light absorption characteristics of the leaf at the two wavelengths are affected by leaf chlorophyll content, the measured values provide an indication of the relative amount of total chlorophyll present in the leaves.

Leaf relative water content was determined from fresh, green and fully expanded leaves from five sample plants, following the procedure of Barrs and Weatherly (1962). Leaves were collected in polyethylene bags and preserved in an ice box. In the laboratory, 2.5 cm × 2.5 cm leaf discs were taken by excluding midribs. Fresh weight (FW) was determined and then, the leaf disc was transferred into a Petri dish containing distilled water, and kept for 24 hours. After 24 hours, the leaf discs were taken out of the distilled water and dried on tissue paper to remove the surface water droplets and immediately weighed to obtain turgid weight (TW). Thereafter, they were oven dried to a constant temperature of 80 °C for 24 hours, and

weighed to get dry weight (DW). The RWC was calculated as:

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

..... Equation 1

Where:

FW = Fresh weight, DW = Dry weight, and TW = Turgid weight

Proline content of the leaves was measured spectrophotometrically from fully expanded leaves, following the Ninhydrin methods of Bates *et al.* (1973), at the Eco-physiology laboratory of Addis Ababa University. The reagent Acid-ninhydrin was prepared by dissolving 1.25 g of ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid.

The weighed fresh leaf sample (0.5 g) was ground using a mortar and pestle and homogenised with 10 ml of 3% sulfosalicylic acid. The homogenate was filtered through Whiteman No. 1 filter paper (Whiteman, Missouri, USA) and the filtrate was used for proline estimation. Two milliliters of filtrate from each sample was taken in separate test tubes; and in each test tube 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid were added and boiled in a hot water bath for one hour. Then, the test tubes were transferred to ice water-bath for one hour to cool, and 4 ml of toluene was added. The mixture was shaken thoroughly and allowed to settle at room temperature for 30 minutes until it formed two separate layers. The upper toluene layer containing the colour complex due to proline ninhydrin reaction was transferred to a separate test tube and absorbance was read in spectrophotometer (Dalian Cebon Instruments Co., Ltd, Liaoning, China) at 520 nm. The proline concentration was determined from the standard curve and

expressed in moles of proline per gram fresh weight of the leaf as:

$$\text{Mole proline g} = \frac{\left(\frac{\mu\text{g proline ml}^{-1}}{\text{ml toluene}}\right) / \left(\frac{115.5 \mu\text{g}}{\mu\text{mole}}\right)}{\left(\frac{\text{g sample}}{5}\right)}$$

..... Equation 2

At maturity, all the panicles from the tagged plants in each plot were harvested, sun dried, threshed, cleaned and weighed. Panicle weight and grain yield were recorded per plot, and later converted to per hectare. The weight of hundred seeds (HSW) was determined from dry seeds at 12.5% moisture level. To obtain shoot dry matter weight (SDW) at maturity, the shoots of the tagged sample plants were harvested and separated into stems and leaves. These parts were dried at 80°C for 24 hours in an oven and dry weights of separated plant parts recorded and summed up.

All the collected quantitative data were subjected to analysis of variance (ANOVA) using STATISTICA Software Version 7 (STATISTICA Inc., USA). Significant means of each measured data were separated using Tukey's multiple range test at the probability of 5%. Correlation coefficients were computed among the studied parameters using SAS version 9.0 Software.

RESULTS

Morphological characters. There were significant difference ($P < 0.05$) among the genotypes for leaf senescence rating under the two water regimes (Table 1). As expected, B35 and E36-1 had the lowest leaf senescence rating (least sensitive) of all the parental lines. On the contrary, Gambella 1107 showed the highest rating (the most sensitive) under stressed condition. Meko/B35-12, Gambella/B35-5 and Teshale/E36-1 showed the lowest

TABLE 1. Effect of post flowering drought stress on leaf senescence rating (LSR), leaf chlorophyll content (LCC, $\mu\text{g cm}^{-2}$), and relative water content (RWC, %) of sorghum genotypes in Ethiopia

Genotypes	LSR*		LCC		RWC	
	WW	DS	WW	DS	WW	DS
Teshale/B35	2.89 ^{dfg}	3.31 ^{abcd}	52.94 ^{ab}	44.08 ^{abcde}	78.37 ^{abc}	53.07 ^g
Teshale/E36-1	2.72 ^f	3.11 ^b	42.66 ^{abcde}	41.99 ^{abcde}	85.75 ^a	67.59 ^c
Gambella 1107/E36-1	2.88 ^{dfg}	3.28 ^{abcd}	37.38 ^b	31.86 ^d	79.59 ^{abc}	56.97 ^f
Gambella 1107/B35-code1	2.96 ^{dfg}	3.38 ^{abc}	30.71 ^{de}	27.54 ^e	76.83 ^{abcd}	53.39 ^g
Gambella 1107/B35-code5	2.83 ^{dfg}	3.08 ^c	41.43 ^{abcde}	40.99 ^{abcde}	85.71 ^a	61.92 ^e
Meko/B35-code8	2.90 ^{dfg}	3.31 ^{abcd}	46.76 ^{abcd}	41.00 ^{abcde}	74.19 ^{abcde}	56.44 ^{fg}
Meko/B35-code12	2.85 ^{dfg}	3.06 ^d	38.47 ^{abcde}	36.28 ^{bce}	84.65 ^{ab}	64.26 ^d
Teshale	2.78 ^c	3.58 ^{ab}	50.25 ^{abc}	36.76 ^{bcde}	85.7 ^a	52.94 ^g
Gambella 1107	2.86 ^{dfg}	3.64 ^a	51.64 ^{abc}	38.92 ^{abcde}	80.74 ^{abc}	53.03 ^g
Meko	2.88 ^{dfg}	3.58 ^{ab}	44.28 ^{abcde}	33.73 ^c	82.66 ^{ab}	53.07 ^g
E36-1	2.50 ^g	2.57 ^g	49.75 ^{abc}	43.53 ^{abcde}	83.96 ^{ab}	74.66 ^{abcde}
B35	1.34 ^h	1.36 ^h	56.11 ^a	47.66 ^{abcd}	83.05 ^{ab}	71.37 ^b

*WW = well-watered, DS = drought stressed

rate of senescence of all the introgression lines, whereas, Gambella 1107/B35-1, Teshale /B35 and Meko/B35-8 showed the highest leaf senescence rating. Under the stressed condition, all of the introgression lines showed lower leaf senescence rating than their recurrent parents (Table 1).

Physiological and biochemical characters

Relative water content. There were significant differences ($P < 0.05$) among the genotypes for leaf RWC under the two water regimes (Table 1). All the genotypes showed a reduction in RWC due to drought stress. Under drought stressed condition, E36-1, B35 and Teshale/E36-1 showed the highest RWC in that order. The recurrent parents, Meko, Gambella 1107 and Teshale possessed the lowest leaf RWC of all the genotypes under drought stressed condition. However, all of the introgression lines had higher RWC than their recurrent parents.

Chlorophyll. There were significant differences ($P < 0.05$) in chlorophyll content among the genotypes (Table 1). Leaf chlorophyll content (SPAD values) of the

genotypes differed between the two water regimes, and post-flowering drought significantly reduced the leaf chlorophyll content of all the genotypes. Under drought stressed condition, B35, Teshale/B35, E36-1, Teshale/E36-1 and Meko/B35-8 maintained the highest chlorophyll content; while Teshale, Meko/ B35-12, Meko, Gambella 1107/E36-1 and Gambella 1107/B35-1 had the lowest values of this parameter (Table 1). Chlorophyll content of the leaves among genotypes ranged from $30.71 \pm 1.66 \text{ mg cm}^{-2}$ for Gambella 1107/B-35-1 to 47.66 mg cm^{-2} for B35 under drought stressed condition. Under the stressed condition, all introgression lines, except crosses of Gambella 1107 (Gambella 1107/B35-1 and Gambella 1107/E36-1), had higher chlorophyll content than their recurrent parents (Table 1).

Transpiration. Transpiration rate also showed significant difference ($P < 0.05$) among the genotypes under the two water regimes (Table 1). Rate of transpiration was significantly lower under water stressed treatments than with irrigation, and showed a general decreasing pattern with drought stress. Under the irrigated condition, Teshale/B35 had

the highest transpiration rate; followed by Teshale/E36-1 and Gambella 1107/E36-1. The lowest transpiration rate was recorded by B35 under both water regimes. On the other hand, the maximum transpiration rate under water limited condition was observed in Teshale/B35 (Table 2). Under the water stressed condition, all of the introgression lines had higher transpiration rate than their recurrent parents.

Water use efficiency. Significant differences ($P < 0.05$) were observed among the genotypes for WUE under the two contrasting water regimes (Table 2). E36-1, Gambella 1107/B35-5 and B35 had the highest WUE of all the genotypes under drought stressed condition. The two most water use efficient stg introgression lines were Teshale/E36-1 and Gambella 1107/B35-5. All of the introgression lines, except Gambella 1107/B35-5 and Meko/B35-8 had higher WUE than their recurrent parents under drought stressed condition (Table 2).

CO₂ assimilation. There were significant variations ($P < 0.05$) among the genotypes for

CO₂ assimilation rate under the two water regimes (data not shown). A decrease in CO₂ uptake was observed in all the genotypes under drought stressed condition. Under this condition, Gambella 1107/B35-5 and Teshale/E36-1 had slightly superior assimilation rates than the rest of the genotypes. Under well-watered condition, all of the introgression lines had lower assimilation rate than their respective recurrent parents; but under drought stressed condition, all of the introgression lines except Meko/B35-8 and Gambella 1107/B35-1, had higher assimilation rate than their recurrent parents (Table 2).

Proline content. Proline content showed significant difference ($P < 0.05$) among the genotypes under both water regimes (Table 2). All genotypes increased in level of proline under drought stress. The level of increase ranged between 79.4% (B35) and 86.3% (E36-1). Under water stressed condition, Teshale/E36-1 had the highest proline content followed by E36-1 and Gambella/B35-5 (Table 2). Maximum proline content was observed in better yielding lines, Teshale/E36-1,

TABLE 2. Effect of post-flowering drought stress on CO₂ assimilation rate, transpiration rate, water use efficiency and proline content of sorghum genotypes in Ethiopia

Genotypes	AR*		TR		WUE		Prol	
	WW	DS	WW	DS	WW	DS	WW	DS
Teshale/B35	1.99abcde	0.61 ^{cde}	2.95 ^a	2.00 ^{bcd}	0.78 ^{abc}	0.35 ^c	0.52 ^f	2.67 ^{cd}
Teshale/E36-1	2.41abc	1.95 ^{abcde}	2.77 ^{ab}	1.45 ^d	0.94 ^{abc}	1.50 ^{abc}	0.54 ^e	3.74 ^a
Gambella 1107/E36-1	2.21abcde	0.79 ^{bcd}	2.39 ^{abc}	1.38 ^{de}	1.28 ^{abc}	0.65 ^{abc}	0.53 ^e	2.78 ^c
Gambella 1107/B35-code1	2.08abcde	0.68 ^{cde}	1.96 ^c	1.28 ^e	1.04 ^{abc}	0.63 ^{abc}	0.54 ^e	2.67 ^{cd}
Gambella 1107/B35-code5	2.57ab	1.91 ^{abcde}	1.32 ^e	1.26 ^e	2.00 ^{abc}	1.97 ^{abc}	0.55 ^e	3.49 ^{abc}
Meko/B35-code8	1.65abcde	0.61 ^{cde}	1.67 ^{cde}	1.03 ^{fg}	1.28 ^{abc}	1.20 ^{abc}	0.51 ^f	2.69 ^{cd}
Meko/B35-code12	2.17abcde	0.78 ^{bcd}	2.12 ^b	1.41 ^{de}	1.08 ^{abc}	0.82 ^{abc}	0.53 ^e	3.36 ^{abc}
Teshale	2.72a	0.55 ^d	1.11 ^{fg}	0.64 ^{fg}	2.70 ^b	0.86 ^{abc}	0.51 ^g	2.64 ^{cd}
Gambella 1107	2.85a	0.74 ^c	1.44 ^{de}	0.88 ^{fg}	2.09 ^{abc}	1.10 ^{abc}	0.52 ^e	3.07 ^b
Meko	2.31abcd	0.66 ^{cde}	1.24 ^{ef}	0.71 ^{fg}	1.94 ^{abc}	0.93 ^{abc}	0.52 ^e	3.06 ^{bc}
E36-1	2.27abcd	0.86 ^b	1.17 ^f	0.46 ^{fg}	2.37 ^a	2.33 ^{abc}	0.51 ^g	3.73 ^{ab}
B35	1.46abcde	0.44 ⁺	0.83 ^{fg}	0.37 ^g	1.89 ^{abc}	1.29 ^{abc}	0.50 ^g	2.43 ^d

*WW = well-watered, DS = drought stressed, AR = assimilation rate (g cm⁻²); TR = transpiration rate (mg m⁻²/s); WUE = water use efficiency; Prol = proline content (imol g⁻² fresh weight)

Gambella 1107/B35-5 and Meko/B35-12, and one of their *Stg* donor parents (E36-1) whereas the minimum was in Teshale and B35.

Yield and yield related traits. Shoot dry weight (SDW) showed significant differences ($P<0.05$) among the genotypes under the two water regimes (data not shown). All of the genotypes had higher SDW under well-watered regime than under water limited condition. Under the stressed condition, Teshale/E36-1 and Gambella 1107/B35-5, had the highest; and B35 had the lowest SDW of all the genotypes. Shoot dry weight among the genotypes ranged from 340.15 ± 7.10 g (B35) to 541.12 ± 14.91 g (Teshale/E36-1) and from 335.68 ± 13.86 g (B35) to 537.18 ± 12.39 g (Teshale/E36-1) under well-watered and drought stressed conditions, respectively. Under the drought stressed condition, all of the introgression lines except Gambella 1107/E36-1 and Gambella 1107/B35-1 had higher SDW than their recurrent parents (Table 3).

There was a significant difference ($P<0.05$) among the genotypes for grain yield, panicle weight and hundred seed weight under the two water regimes (Table 3). A general reduction

in panicle weight was observed in all the genotypes, related to drought stress. Under drought stressed condition, Teshale/E36-1, Gambella 1107/B35-5 and E36-1, in that order, showed the highest panicle weight; while, Gambella 1107/B35-1 showed the lowest panicle weight. Under well-watered condition, all of the introgression lines had lower panicle weight than their recurrent parents; but under stressed condition, only four of the seven introgression lines, Teshale/E36-1, Gambella 1107/B35-5, Meko/B35-12 and Meko/B35-8, showed lower panicle weight than their recurrent parents (Table 3).

Due to post-flowering drought stress condition, the 100 seed weight (HSW) of the genotypes ranged from 2.18 ± 0.12 g (B35) to 3.65 ± 0.19 g (Teshale/E36-1). Under the well-watered condition, all of the introgression lines had lower HSW than their recurrent parents; but under the stressed condition, all of the introgression lines, except Gambella 1107/B35-1, Meko/B35-8 and Teshale/B35, had HSW either higher or not significantly different from their recurrent parents (Table 3).

Grain yield of all genotypes exposed to post-flowering drought stress was lower than those

TABLE 3. Effect of post-flowering drought stress on shoot dry weight, panicle weight, hundred seed weight, and grain yield for sorghum genotypes in Ethiopia

Genotypes	SDW*		PW		HSW		GY	
	WW	DS	WW	DS	WW	DS	WW	DS
Teshale/B35	514.99 ^{abc}	461.37 ^{de}	42.45 ^{de}	34.77 ^e	2.78 ^e	2.25 ^h	2.75 ^{abcdef}	1.97 ^{ef}
Teshale/E36-1	541.12 ^a	537.18 ^a	81.46 ^{abc}	76.63 ^{abcd}	3.80 ^{abc}	3.65 ^{abcde}	3.85 ^{abc}	3.65 ^{abc}
Gambella 1107/E36-1	432.10 ^e	425.06 ^e	57.55 ^d	36.71 ^{de}	3.02 ^b	2.66 ^f	3.39 ^{abcdef}	2.87 ^{abcdef}
Gambella 1107/B35-code1	423.31 ^e	405.34 ^e	46.65 ^{bc}	25.83 ^f	3.00 ^c	2.55 ^{fgh}	3.00 ^{bcdef}	2.74 ^{abcdef}
Gambella 1107/B35-code5	531.52 ^{ab}	526.49 ^{abc}	76.99 ^{abcd}	71.75 ^{abcde}	3.71 ^{abcd}	3.55 ^{abcde}	3.89 ^{ab}	3.60 ^{abcd}
Meko/B35-code8	452.89 ^{de}	425.08 ^e	56.40 ^{abcde}	44.34 ^{be}	2.89 ^d	2.38 ^g	2.51 ^{bcdef}	2.02 ^e
Meko/B35-code12	473.89 ^b	467.74 ^c	67.15 ^{abcde}	60.58 ^{abcde}	3.36 ^{abcdef}	3.02 ^{bcdefgh}	3.28 ^{abcdef}	3.29 ^{abcdef}
Teshale	539.30 ^a	456.27 ^{de}	85.78 ^{ab}	52.46 ^b	3.90 ^{ab}	2.58 ^{fgh}	4.04 ^{ab}	2.03 ^{def}
Gambella 1107	527.13 ^{abc}	463.57 ^d	89.42 ^a	54.84 ^{abcde}	3.91 ^a	2.60 ^{fgh}	4.23 ^a	2.52 ^b
Meko	449.89 ^e	413.50 ^e	68.93 ^{abcde}	43.84 ^c	3.58 ^{abcde}	2.56 ^{fgh}	3.74 ^{abc}	2.28 ^c
E36-1	431.83 ^e	428.60 ^e	70.47 ^{abcde}	63.25 ^{abcde}	3.41 ^{abcdef}	3.17 ^{abcdefg}	3.48 ^{abcde}	3.16 ^{abcde}
B35	340.15 ^f	335.68 ^f	40.09 ^{ce}	34.63 ^e	2.31 ^{gh}	2.18 ^h	2.03 ^d	1.89 ^f

*WW = well-watered; DS = drought stressed; SDW = shoot dry weight (gm); PW = panicle weight (gm); HSW = hundred seed weight (gm); GY = grain yield ($t\ ha^{-1}$)

under well-watered condition (Table 3). Grain yield ranged from 2.03 t ha⁻¹ (B35) to 4.23 t ha⁻¹ (Gambella 1107) under well-watered condition and from 1.89 t ha⁻¹ (B35) to 3.65 t ha⁻¹ (Teshale/E36-1) under drought stress growing condition. Under well-watered condition, all the introgression lines had lower grain yield than their recurrent parents, but under water limited condition, all the introgression lines, except Meko/B35-8 and Teshale/B35, had higher grain yield than their recurrent parents.

Correlations among the measured parameters. The Pearson's coefficient of correlation among the various parameters measured under drought stressed condition showed that CO₂ assimilation rate had a significant positive correlation with WUE ($r=0.63$), proline content ($r=0.56$), HSW ($r=0.55$), SDW ($r=0.62$) and grain yield ($r=0.52$). Moreover, proline content had a very strong positive correlation with HSW ($r=0.74$), SDW ($r=0.59$), panicle weight ($r=0.72$), and grain yield ($r=0.70$). The correlation of SDW ($r=0.64$), panicle weight ($r=0.70$) and with grain yield ($r=0.68$) was very strong and positive. Maximum proline content was observed in better yielding lines, Teshale/E36-1, Gambella 1107/B35-5 and Meko/B35-12, and one of their *Stg* donor parents (E36-1) whereas the minimum was in Teshale and B35.

DISCUSSION

Stay green trait. Leaves of the recurrent parents senesced more than both the donor parents and their introgression lines under induced drought condition (Table 1). Similarly, high leaf senescence under post-flowering drought condition was reported by Farooq *et al.* (2009) and Bezabih (2012). Water deficit reduces individual leaf size and leaf longevity by increasing leaf senescence and by decreasing the soil's water potential (Anjum *et al.*, 2011a). However, the response varies depending on the genotype's ability to maintain green leaf area.

A relatively lower leaf senescence rating was observed in *Stg* donor lines, B35 and E36-1, and their QTL introgression lines Meko/B35-12, Gambella 1107/B35-5 and Teshale/E36-1, under drought stressed condition (Table 1), perhaps due to the *Stg* trait. The higher rate of maintenance of green leaf area in B35 was in agreement with Kassahun (2006) who compared different genotypes of sorghum for drought tolerance in relation to stay green trait. The *Stg* trait results in greater functional photosynthetic leaf area during grain filling and after physiological maturity (Sanchez *et al.*, 2002). The higher leaf senescence rating in the recurrent parents, Gambella 1107, Meko and Teshale, under stressed condition could be due to absence of *Stg* trait. This may also suggest that the induced water deficit sufficiently severed to differentiate the senescent genotypes from non-senescent (*Stg*) ones. Previous reports also suggested that drought stress during the post-flowering period accelerates senescence of non-*Stg* sorghum genotypes (Wingler *et al.*, 2006; Borrell *et al.*, 2014).

Physiological parameters. In the present study, it has been found that induced post-flowering water deficit decreases chlorophyll content of all the test genotypes (Table 1). This was in agreement with previous studies (Mostafa *et al.*, 2011; Sharada and Naik, 2011). In line with the result of the present study, Khayatnezhad and Gholamin (2012) reported that chlorophyll content of both resistant and sensitive cultivars reduced under drought stress.

The reduction in chlorophyll content under drought stressed condition could be due to thylakoid membrane damage, caused by increased production of reactive oxygen species (Kapanigowda *et al.*, 2013). It has been reported that post-flowering drought resistant genotypes have higher chlorophyll content than susceptible genotypes (Arjenaki *et al.*, 2012). Such phenomenon of retaining higher chlorophyll content under water deficit condition serves as a water deficit stress

tolerance mechanism in sorghum genotypes with *Stg* property (Thomas and Howarth, 2000). Similarly, in the present study, B35, Teshale/B35, E36-1 and Teshale/E36-1 maintained the highest chlorophyll content under water deficit condition and can be tentatively considered as tolerant to post flowering drought stress. In addition, high chlorophyll content indicates a low degree of photoinhibition of photosynthetic apparatus and is, thus desirable (Almeselmani, 2011). On the other hand, a decrease in chlorophyll content with drought stress implies a lowered capacity for light harvesting (Mafakheri *et al.*, 2010) due to the reduced efficiency of light capturing to drive photosynthesis (Anjum *et al.*, 2011b).

Relative water content (RWC) is considered as a measure of plant water status, reflecting the metabolic activity in tissues and serves as the most meaningful measurements for drought tolerance. In the present study, drought stress brought about a dramatic decline in RWC in all the genotypes (Table 1). This was in agreement with the previous reports (Keyvan, 2010; Unyayar *et al.*, 2004; Xu *et al.*, 2000) that induced drought stress in sorghum significantly affected RWC. This reduction might be triggered by water deficit in the soil and/or as a consequence of water loss *via* the stomata (Keyvan, 2010). Under the drought stressed condition, the *Stg* donor parents (B35 and E36-1) maintained the greatest mean RWC of all genotypes (Table 1) perhaps because *Stg* character helped them to keep the stalk transportation system functioning under this conditions (Xu and Zhou, 2008). The *Stg* trait resulted in decreased water use early in the season and allowed water to be conserved to sustain a longer period of grain filling (Kapanigowda *et al.*, 2013). Teshale/ E36-1, Meko/B35-12, and Gambella 1107/B35-5 had superior RWC to the other introgression lines, and recurrent parents under the drought stressed condition. Genotypes that keep their stomata open under the drought stress conditions and maintain adequate RWC can be in general considered as suitable for dry

regions (Krouma, 2010). In this regard, the above mentioned genotypes could be suitable for dry regions of Ethiopia.

In general, introgression lines and their parents showed a decline in assimilation rate under post-flowering drought stress (Table 2). This was in agreement with previous reports (Griffiths and Parry, 2002; Krouma, 2010). Assimilation rate is particularly affected by water deficiency, which causes functional and structural rearrangements of photosynthesizing apparatus (Griffiths and Parry, 2002). On the other hand, the observed decline in assimilation rate might be due to a decrease in RWC, which leads to turgor loss of guard cells, causing passive stomatal closure. This leads to a decrease in CO₂ uptake and lets the plants consume a lot of energy to absorb water, which in turn causes a reduction in photosynthesis (Valadabadi *et al.*, 2009).

The decline in the rate of CO₂ uptake was observed in the studied genotypes, except Teshale/E36-1 and Gambella 1107/B35-5 (Table 2). However, Teshale/E36-1, B35 and Gambella 1107/B35-5 showed less sensitivity to drought stress condition, implying their better tolerance to drought stress than the rest of the tested genotypes. The comparable photosynthetic performance of these lines with the known post-flowering drought resistant donor parents (B35 and E36-1), could be evidence of better assimilation rate under severe water deficit conditions. In contrast, the higher sensitivity of Teshale to declining soil moisture as evident from the lowest CO₂ uptake shows its sensitivity to drought stress. This observation suggests that the higher chlorophyll content of Teshale/B35 and Meko/B35-8 was not associated with high assimilation rate. Therefore, maintenance of green leaf area in Teshale/B35 and Meko/B35-8 was probably cosmetic or non-functional type (Thomas and Howarth, 2000).

Even though, in general terms, sorghum genotypes possessing the *Stg* trait retain green stems and leaves continue to photosynthesize even under terminal drought stress than those

that do not have this trait (Harris *et al.*, 2007), this happens only if *Stg* is associated with assimilation rate, which is functional *Stg*. Thus, assimilation rate is a useful criterion in differentiating drought tolerant genotypes from sensitive ones; and those with cosmetic and non-cosmetic/ functional *Stg* types.

In conformity with previous studies (Mafakheri *et al.*, 2010; Kapanigowda *et al.*, 2013), genotypes revealed a general trend of reduction in transpiration rate under the drought stressed condition (Table 2). The transpiration rates of genotypes under the well-watered condition were higher than those under stressed condition. Stomatal closure is probably responsible for the decline in transpiration rate in tolerant genotypes, under the drought stressed condition, as it is the most efficient way of reducing transpirational water loss (Yordanov *et al.*, 2001). In contrast, Teshale/B35 and Teshale/E36-1 had higher transpiration efficiency under the drought stressed condition, which might be due to the presence of the *Stg* trait. Similarly, Borrell *et al.* (2003) reported that some genotypes with *Stg* characteristics possessed enhanced transpiration efficiency, which enable the plant to set a higher yield potential during anthesis, which ultimately leads to higher grain yield.

E36-1, Gambella 1107/B35-5 and B35 had higher WUE, which is an indication of improved drought tolerance by optimising CO₂ uptake per unit of water lost through transpiration. This was possibly done through maintaining better RWC and opening of stomata partially, which allowed a relatively higher assimilation and a reduced transpiration (Cornic, 2000). Van Oosterom *et al.* (2006) suggested *Stg* to be the consequence of water saving from having higher WUE. Furthermore, the reduced WUE of Teshale/B35 and Gambella 1107/B35-1 was perhaps due to extensive stress-induced leaf senescence. Therefore, WUE indicates the tissue water relation of sorghum lines and their parents and suggests the difference in adaptation strategies among the genotypes to drought stress.

Proline content. Leaf proline content of the tested introgression lines and their parents under water stressed condition showed significant variation (Table 2). The minimum proline content in the *Stg* donor line, B35, though difficult to explain, may indicate that the induced drought was not severe for it to accumulate high proline. It is known that proline is among the several osmolytes, whose accumulation increases when the plants experience some kind of stress such as drought and salt as a defensive mechanism (Reddy *et al.*, 2015). Accumulation of proline under the stress condition was reported to have correlated with stress tolerance, and its concentration has been shown to be generally higher in stress tolerant than in stress sensitive plants (Pirdashti *et al.*, 2009; Sharada and Naik, 2011).

Yield and yield related traits. A significant reduction in SDW under drought stress condition was observed in all of the genotypes studied (Table 3), which was in agreement with Valadabadi *et al.* (2009) and Anjum *et al.* (2011a). The observed reduction in SDW was perhaps due to the reduction in photosynthesis as a result of leaf area reduction, decrease in chlorophyll synthesis and the rise in the energy consumed by the plant in order to take in water (Valadabadi *et al.*, 2009). The effect of drought stress is mainly observed in the form of decreasing in SDW (Hamayun, 2010). The maximum SDW was recorded by Teshale/E36-1, Gambella 1107/B35-5 and Meko/B35-12; while the minimum SDW was recorded by B35 under the stressed condition (Table 3). Sorghum genotypes with *Stg* trait had higher shoot dry matter accumulation at maturity, indicating that grain filling occurs because of current photosynthesis rather than remobilisation from stem reserves, which was in agreement with the results of Renuka and Chimmad (2006). This is expected because SDW production and accumulation in plants is mainly the result of photosynthesis, which requires water (Okiyo *et al.*, 2008). The

variation in SDW under drought stress conditions would help screen tolerant genotypes from sensitive as tolerant genotypes have less reduction in shoot dry matter than susceptible ones under water deficit stress conditions (Pace *et al.*, 1999).

Post-flowering drought stress caused reduction in grain yield and panicle weight in all of the genotypes (Table 3). The reduction in grain yield was the highest for Teshale followed by Gambella 1107 and Meko, all of which were susceptible recurrent parents. This was in agreement with the reports of Asgharipour and Heidari (2011), who found significant reduction in grain yield. Moreover, Tuinstra *et al.* (1997) reported that the effect of post-flowering drought stress on sorghum grain yield was significant compared to well-watered condition. The reduction of grain yield of sorghum genotypes could be a result of fewer seeds per unit area and smaller size of the panicle (Ibrahim *et al.*, 2013). It was also clear that Teshale/E36-1 and Gambella 1107/B35-5 had superior grain yield and panicle weight under drought stress to the *Stg* donor parent (E36-1) (Table 3). The *Stg* donor parent B35 had the lowest grain yield overall, which was in line with the reports of Adugna and Tirfessa (2014) and Kassahun *et al.* (2010), who reported that B35 was generally low yielder due to its small panicle size and low grain number per panicle. On the other hand, the observed high grain yield in Teshale/E36-1 and Gambella 1107/B35-5 was consistent with relatively higher assimilation rate, high panicle weight, and HSW under stressed condition (Table 3).

Low transpiration rate in E36-1 that resulted in higher WUE (Table 2) could have favoured high assimilation, ultimately better grain yield under drought stress. The lowest grain yield of B35, Teshale/B35 and Meko/B35-8 was also reflected in parallel decline in assimilation rate. This might be due to the fact that grain development is related to assimilation rate during anthesis and is sensitive to water

stress (Tuinstra *et al.*, 1997), which might have caused reduced HSW. Similar to this study, Kassahun (2006) found significantly lower biomass, grain size and grain yield in the majority of B35 sorghum backcross derivatives to their elite recurrent parents, which were attributed to the effect of undesirable linkage drag.

A general reduction in HSW was observed among lines and their parents under water stressed condition (Table 3), which was in agreement with the results of Rosenow and Clark (1995). This might be as a result of the decline in photosynthetic capacity and lack of enough assimilates to fill the developing grains (Tuinstra *et al.*, 1997). Under the stressed condition, some of the *Stg* introgression lines had higher grain weight than their senescent recurrent parents (Table 3), which suggests the contribution of *Stg* QTLs for continued photosynthesis and accumulation of assimilates under post-flowering drought condition.

Relationship among parameters. Proline content had significant positive correlation with assimilation rate and grain yield under drought stress (Table 1). The positive and significant correlation of proline content with other measurements proves its importance as a selection criterion for *Stg* QTL introgression lines under post-flowering drought stress. The correlation between AR and GY under stress was in conformation with previous reports in rice (Pirdashti *et al.*, 2009; Kumar *et al.*, 2014) and in bread wheat (Keyvan, 2010).

Hundred seed weight (HSW) showed significant positive correlation with SDW, panicle weight (PW) and grain yield (Supplementary Table). This confirms that HSW, SDW and PW are the components of grain yield. Moreover, the above correlations indicate the potential and relevance of physiological measurements in determining *Stg* property and drought tolerance in sorghum genotypes. Thus, proline content, CO₂ assimilation and HSW could be useful in

determining yield of sorghum under post-flowering drought stress, and are fairly effective parameters for evaluation of drought tolerance.

CONCLUSION

The present study indicates the existence of variability among *Stg* QTL introgression sorghum lines developed through molecular marker assisted breeding in 2006 - 2008 in Ethiopia, in terms of their reaction to measured parameters under post-flowering drought stress. The following *Stg* QTL introgression sorghum lines were selected based on their drought stress tolerance and having relatively higher yield potential under drought: Teshale/E36-1, Gambella 1107/B35-5 and Meko/B35-12. Two of the lines: Teshale/E36-1 and Meko/B35-12 were also found to show better performance in our previous study. The results also showed that *Stg* QTL introgression sorghum lines had a remarkable variation for contrasting characters in response to terminal drought conditions. Based on the present study, proline content, HSW and CO₂ assimilation are recommended as screening tools for *Stg* property, due to their strong correlation with other measurements. The response of the genotypes should be further assessed in the actual multi-location sorghum growing areas that are affected by terminal moisture stress, in order to investigate the effect of QTL x Environment interaction.

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