Unraveling the genetic basis of drought tolerance in potato

Biructawit Bekele Tessema (✉ biructawit.tessema@gmail.com)

Aarhus University <https://orcid.org/0000-0002-5561-4777>

Gebremedhin Gebregiorgis

Ethiopian Institute of Agricultural Research

Richard Visser

Wageningen Universiteit

C. Gerard van der Linden

Wageningen Universiteit

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Abstract

Background: Potato is highly valued as a food security crop but is sensitive to drought stress. A relatively small reduction of the optimum amount of water can already result in a significant reduction in tuber yield. Therefore, unravelling the genetic basis of drought tolerance is important in order to enhance tolerance to drought in potato. We have evaluated a diploid potato backcross mapping population for drought tolerance under field conditions. Water application was withheld at the stage of tuber initiation. At the end of the drought stress period, we collected data on a number of morphological and physiological traits.

Results: The potato population showed significant reduction in several growth traits measured, as well as in tuber yield. We applied quantitative trait locus (QTL) analysis to find genomic regions underlying these drought tolerance traits. We found 60 QTL for several of the traits measured under well-watered and drought stress conditions. From the total number of QTLs identified, 21 were detected under water stress conditions while 39 QTLs were identified under well-watered conditions. Drought specific QTLs for tuber fresh yield was detected on chromosome 7, 9 and 12.

Conclusions: There is genetic variation in potato for drought tolerance and QTLs discovered for drought tolerance traits in this study can aid in improving potato for drought tolerance.

Background

Potato is one of the most important non-grain food commodities worldwide. Potato has a shallow root system in which about 85% of the total root length is concentrated in the upper 0.3 m of soil, making potato sensitive to water shortage [3, 4]. However under well-watered conditions, potato stands out as an efficient water user compared to wheat, maize and rice producing significantly higher kcal per m³ water [5]. The yield losses for potato due to drought stress are expected to increase, since water scarcity is predicted to be severe in most agricultural production areas in the coming decades [6]. Thus, a better understanding of drought responses helps in improving potato for water stress.

Plant response to drought stress is complex and involves several morphological and physiological adaptations. The response of water stress effects on potato depends on the phenological timing, duration and severity of the stress [7]. Water shortage during tuber initiation stage of potato has a drastic effect on tuber yield and makes recovery even more difficult [8]. Many authors have studied drought responses in potato in order to understand the underlying mechanisms of drought stress tolerance[9]. The effect of water scarcity in potato ranges from reduced photosynthetic rate to induced canopy senescence with negative consequences on tuber yield and quality [10, 11].

The availability of genetic variation in wild potato germplasm allows for drought stress tolerance improvements [12, 13]. Such landraces are better adapted to harsh environments including water scarce conditions [12], and genes from this germplasm could help improving cultivated potatoes that are considered susceptible to drought stress [14]. Drought tolerance is a quantitative trait controlled by a

number of genes and/or gene families. The mechanisms underlying drought tolerance in cultivated potato can be studied using progenies derived from interspecific crosses between drought tolerant and drought sensitive potato genotypes.

However, there are only few studies describing the dissection of the genetic basis of drought tolerance in potato [13, 15, 16]. Drought tolerance studies under in-vitro and green house conditions have led to the discovery of QTL for drought tolerance traits in diploid mapping populations, and some of the QTL were drought specific [13, 15]. In the study of [16] QTL were detected for drought tolerance traits both under green house and field conditions with a potential to contribute to potato improvement.

In the present study, a diploid potato backcross mapping population was used to dissect drought tolerance under field conditions. In a semi-arid environment, this potato population was exposed to drought stress starting from tuber initiation and drought tolerance traits were collected during the water stress period. QTL analysis was then applied to find the genomic regions controlling these drought tolerance traits.

Results

Response to drought stress

The CxE potato mapping population showed a significant reduction in growth and yield in response to drought stress conditions (Table 2). The relative reduction of the measured traits due to water shortage ranged from 8 to 67%. In both experiments, tuber weight was highly reduced (67 and 57%, respectively) in response to water stress, showing the significant impact of water shortage on tuber yield. Similarly, biomass production was reduced by half in response to drought stress. Plant height was reduced by 26.1 and 22.6% in 2010 and 2011 experimental years, respectively. The lowest relative reduction in both experimental years due to drought stress was observed for stem numbers, indicating drought stress has little effect on stem number once stems are formed.

In two successive field experiments, analysis of variance revealed highly significant variation for almost all traits measured under drought stress and control conditions (Table 1). Highly significant variation ($P < 0.001$) among genotypes was observed for the above ground growth traits stem number, plant height, shoot fresh and dry weight under drought stress and well-watered conditions. Statistically significant variation was observed for below-ground traits root fresh and dry weight, and root length for both treatment conditions. Total fresh and dry biomass produced under drought stress conditions showed highly significant genotypic differences. Genotypes under water-limited conditions were significantly different for tuber number and tuber fresh and dry weight. The CxE genotypes were significantly different for harvest index based on dry weight only under well-watered conditions. Genotypes also showed significant variation for chlorophyll content measured at 19, 29 and 34 DAS, under both treatment conditions.

Table 1. Mean values of traits measured at harvest both under drought stress and well-watered conditions in two different years with their respective significance value, relative reduction (RR), and broad sense heritability (H²).

Traits	Year	Drought stress			Well-watered			RR (%)
		Mean	Pvalue	H ²	Mean	Pvalue	H ²	
Stem number	2010	2.6	<0.001	0.52	3.3	0.002	0.45	15.00
	2011	5.5	<0.001	0.65	6.1	<0.001	0.56	8.01
Plant height (cm)	2010	26.5	<0.001	0.62	40.2	<0.001	0.76	26.14
	2011	28.1	<0.001	0.77	37.8	<0.001	0.80	22.59
Shoot fresh weight (g)	2011	97.6	<0.001	0.61	233.8	<0.001	0.76	46.47
Shoot dry weight (g)	2011	14.3	<0.001	0.68	31.1	<0.001	0.71	42.15
Root fresh weight(g)	2011	13.7	0.003	0.42	19.6	<0.001	0.77	22.14
Root dry weight(g)	2011	1.8	<0.001	0.65	2.7	<0.001	0.66	17.38
Root length (cm)	2011	14.8	<0.001	0.94	23.4	0.003	0.42	37.07
Root:shoot (dry weight)	2011	0.14	0.017	0.35	0.09	<0.001	0.58	-64.11
Total fresh biomass (g)	2011	266.5	<0.001	0.70	654.3	<0.001	0.91	55.44
Total dry biomass (g)	2011	53.7	<0.001	0.51	114.9	<0.001	0.80	41.14
Tuber number	2010	7.9	<0.001	0.62	16.8	<0.001	0.75	44.57
	2011	14.1	<0.001	0.50	22.0	<0.001	0.61	15.81
Tuber fresh weight(g)	2010	88.2	<0.001	0.53	369.3	<0.001	0.68	67.00
	2011	168.1	<0.001	0.64	420.4	<0.001	0.96	56.91
Tuber dry weight (g)	2011	39.1	0.034	0.30	83.7	<0.001	0.74	34.51
Harvest index dry weight	2011	0.73	0.404	0.00	0.73	0.011	0.37	-1.20
CC19DAS	2010	49.4	<0.001	0.58	47.1	<0.001	0.69	-5.20
	2011	49.2	<0.001	0.73	48.4	<0.001	0.79	-1.89
CC29DAS	2010	49.8	<0.001	0.54	46.8	<0.001	0.63	-7.00
	2011	45.8	<0.001	0.66	43.2	<0.001	0.78	-6.27
CC34DAS	2010	46.9	0.004	0.44	44.8	<0.001	0.59	-5.58
	2011	44.3	<0.001	0.81	42.7	<0.001	0.82	-3.95

Heritability

Broad sense heritability estimates of traits measured under drought and control conditions are presented in Table 1. The estimates of heritability varied over treatment, and ranged from moderate to high under both treatment conditions in both experimental years. Highest heritability under stress was observed for root length (0.94) and for tuber fresh weight (0.96) under control condition. The heritability for shoot fresh and dry weight under water stress conditions was 0.59 and 0.68, respectively. Under water stress conditions, root to shoot ratio and tuber dry weight had low heritability. A moderate heritability was seen for stem number, root length and tuber dry matter content under well-watered conditions. Heritability was high for chlorophyll content measured in 2011 at all-time points, but decreased in the 2010 experiment with prolonged stress.

Correlations among traits

Phenotypic correlation coefficients for traits measured under well-watered and drought stress conditions are shown in Fig.1. Under stress conditions, plant maturity was positively correlated with shoot fresh weight, shoot dry weight and tuber yield. This implies that genotypes that matured late had higher shoot biomass and tuber yield. Under well-watered conditions plant maturity also showed positive correlations with tuber yield, shoot fresh and dry biomass. Root length, root fresh and dry weight had significant positive correlation with tuber yield indicating genotypes with strong root expansion had better tuber yield. This correlation under well-watered condition was also significant. Moreover, tuber yield under water stress and well-watered conditions showed significant correlation with plant height, total fresh and dry biomass. Remarkably, relatively high correlations were found for tuber number with a number of growth traits under well-watered conditions, while these correlations were much lower under drought stress. On the other hand, tuber number had a higher significant correlation with root length under water stress conditions than under control conditions. Under drought stress conditions, tuber dry weight had significant positive correlations with several of the traits, including plant height, shoot fresh and dry weight, root dry weight, total fresh and dry biomass, and tuber number. Harvest index based on dry weight measured under water limited conditions exhibited a stronger significant negative correlation with root traits than under well-watered conditions. Root to shoot ratio was significantly correlated to harvest index under control conditions, but not anymore under water-limiting conditions, also indicating that the investment in roots did not result in higher tuber yield. The differential response of the root to shoot ratio was illustrated by the relatively poor correlation between control and drought for this trait (0.29). Harvest index had the lowest correlation between drought and control treatment (0.12), indicating a strong treatment by genotype effect. Another trait with relatively poor correlation between drought and control is tuber number (0.31), which was lower than fresh and dry tuber weight (0.71 and 0.56, respectively), which may indicate a specific effect of drought on tuberization while tuber bulking is much less affected.

QTL mapping

A QTL analysis was done to unravel the genetic basis of the phenotypic variation observed in the CxE population under drought stress and well-watered conditions. In two successive field experiments, a total of 60 QTLs were detected for agronomical, morphological and physiological traits (Table 2; Fig.2). These QTLs were found on all the different chromosomes, except on chromosome 4. Most of the QTLs detected were for the 2011 experiment since several of the traits were measured for this year only. For well-watered treatment 39 QTLs were detected while under drought stress conditions, 21 QTLs were found. The phenotypic variation explained by the QTLs detected under water stress conditions ranged from 7.5 to 55.2%, while QTLs identified under well-watered conditions explained 7.0 to 45.7% of the phenotypic variations observed. Under both treatment conditions several of the QTLs were located on chromosome 5 and many of the QTLs co-localized in the 26.2-44.0 cM interval, while one QTL identified under well-watered conditions for harvest index (dry weight) was located in the interval 52.4-66.5cM. These two different locations on chromosome 5 were also identified to contain QTL under drought stress and well-watered conditions in a greenhouse experiment [13], and an expression QTL hotspot (40-50 cM) was identified on chromosome 5 for drought stress conditions [17].

QTLs accumulating in the 26.2-44.0cM interval on chromosome 5 included QTLs for plant height under stress in both experimental years. For this trait, a QTL on chromosome 8 was identified for 2010 only that explained 13.7% of phenotypic variation. Further QTLs accumulating in the same region on chromosome 5 included QTLs for shoot fresh weight and shoot dry weight for drought stress treatment with high LOD scores of 16.58 and 17.64, accounting for 52 and 54.2% of phenotypic variation, respectively. Under drought stress treatment, identified QTLs for root fresh weight and dry weight in this region had a LOD score of 10.48 and 13.72, explaining 37.1 and 45.5% of observed phenotypic variation. Moreover, QTLs responsible for total fresh biomass with LOD values of 16.54 and total dry biomass with LOD values of 18.16 were detected under water stress treatment. For tuber fresh weight, QTLs located on chromosome 5, 7, 9 and 12 were detected for stress condition. The highest phenotypic variation (28.6%) was explained by a QTL located in the 26.2-44.0 cM interval on chromosome 5. QTLs found on chromosome 7, 9, and 12 were specific to the drought stress condition.

For the well-watered treatment, we detected QTLs on chromosome 5 for stem number, plant height, shoot fresh weight and dry weight, root fresh and dry weight, root length, total fresh and dry biomass, tuber fresh and dry weight, and tuber number. All these QTLs fall in the interval range of 26-47.3 cM. A QTL for harvest index based on dry weight falls in the interval of 52.4-62.9 cM (Fig.2). This suggests there may be two independent QTL regions located on chromosome 5, in agreement with [13, 17].

Twelve significant QTLs were detected on other chromosomes than chromosome 5 for some of the traits measured under well-watered treatment, specifically on chromosome 1, 3, 6, 7, 9, and 11. A QTL on chromosome 1 was identified for root dry weight with LOD value of 4.71 and accounting for 7% of phenotypic variation. On chromosome 3 and 7 QTLs for tuber number were identified that explained 11.8 and 15.3% of variance, respectively. QTLs for root dry weight, tuber number and tuber fresh weight co-localized on chromosome 8. QTLs for plant height were detected on chromosome 6 for both experimental years. Chromosome 9 had a QTL for stem number with LOD value of 4.72 that explained 15.5% of the observed variation. QTLs for shoot fresh weight and total fresh biomass were co-located on chromosome 11, explaining 10.6 and 9.3% of phenotypic variation, respectively.

Several genomic regions responsible for the variation in chlorophyll content were found on chromosome 1, 2, 3, 7, 10, and 11, for different time points and both under drought stress and well-watered conditions. The QTL detected on chromosome 2 was present across years and treatments. This QTL falls in the interval from 79.9 to 109.3 cM and the phenotypic variation explained ranged from 14.3-27.7%. In addition, a QTL expressed across treatments and years was identified on chromosome 10, located between 20 and 46 cM. For well-watered conditions, a QTL on chromosome 1 was found expressed across years. QTLs on chromosome 3 and 7 were identified for chlorophyll content measured under drought stress conditions, and these explained 13.9 and 9.3% of the phenotypic variation, respectively.

Table 2. QTLs detected for listed traits under drought stress (DS) and well-watered (WW) conditions with their linkage groups (LG), 2- LOD support value, intervals (cM), and explained variation (%).

Trait	year	treatment	QTL name	LG	Marker	LOD	interval	%Variation
Stem number	2010	WW	StNr10	CE5	Mando	5.97	26.0-40.0	21.1
		WW	StNr10	CE9	PotSNP594	4.72	53.5-59.9	15.5
	2011	WW	StNr11	CE5	SPUD237	8.7	33.7-43.0	30.8
Plant height	2010	WW	PIHt10	CE5	PotSNP697	16.18	31.2-47.3	44.9
		WW	PIHt10	CE6	PotSNP486	8.05	71.5-77.0	14.7
	2010	DS	PIHt10	CE5	GP21_2007	9.83	27.0-38.0	33.2
		DS	PIHt10	CE8	PotSNP1067	4.71	1-18.6	13.7
	2011	DS	PIHt11	CE5	SPUD237	16.29	32.2-44.0	51.4
	2011	WW	PIHt11	CE5	PotSNP697	15.75	31.2-46.3	42.8
		WW	PIHt11	CE6	PotSNP150	5.21	75.6-79.1	10.4
Shoot fresh weight	2011	DS	SFW11	CE5	SPUD237	16.58	33.7-43.0	52
	2011	WW	SFW11	CE5	Mando	10.84	31.2-42.0	31.5
		WW	SFW11	CE11	PotSNP100	4.48	25.6-38.8	10.6
Shoot dry weight	2011	DS	SDW11	CE5	SPUD237	17.64	32.7-43.0	54.2
	2011	WW	SDW11	CE5	Mando	10.47	30.8-44.0	37.4
Root fresh weight	2011	DS	RFW11	CE5	Mando	10.48	30.0-39.0	37.1
	2011	WW	RFW11	CE5	Mando	13.8	31.2-41.0	45.7
Root dry weight	2011	DS	RDW11	CE5	Mando	13.72	31.2-37.7	45.5
	2011	WW	RDW11	CE1	PotSNP72	4.71	88.0-114.9	7
		WW	RDW11	CE5	Mando	14.62	32.2-36.7	37
		WW	RDW11	CE8	STM1024	4.86	35.7-37.5	7.5
Root length	2011	DS	RL11	CE5	SUPD237	8.36	31.2-44.0	30.9
	2011	WW	RL11	CE5	Mando	11.29	28.0-38.0	39.3
Total fresh biomass	2011	DS	TFB11	CE5	SPUD237	14.45	32.2-44.0	47.3
	2011	WW	TFB11	CE5	Mando	13.74	31.2-	39.6

						40.4		
		WW	TFB11	CE11	PotSNP100	4.36	22.6 - 38.8	9.3
Total dry biomass	2011	DS	TDW11	CE5	SPUD237	15.61	32.7-44.0	49.6
	2011	WW	TDW11	CE5	Mando	10.7	30.0-43.0	37.7
Tuber number	2010	WW	TuNr11	CE3	PotSNP154	4.93	79.5-96.6	11.8
		WW	TuNr11	CE7	PotSNP712	5.43	20-54.9	15.3
		WW	TuNr11	CE8	STM1024	5.79	26.5-41.4	14.6
	2011	WW	TuNr11	CE5	SPUD237	3.6	26.0-49.5	14.7
Tuber fresh weight	2010	WW	TuNr11	CE8	Stl022	4.31	24.5-41.4	19.2
	2011	DS	TuFwt11	CE5	SPUD237	12.38	31.2-43.0	28.6
		DS	TuFwt11	CE7	PotSNP25	4.84	29.3-44.1	9
		DS	TuFwt11	CE9	PotSNP587	4.83	27.1-35.7	9
		DS	TuFwt11	CE12	E39/M60-30e12	4.98	1-21.6	9.4
		WW	TuFwt11	CE5	Mando	9.52	30.0-43.0	34.4

Table 2 continued

Trait	year	treatment	QTL name	LG	Marker	LOD	interval	%Variation
Tuber dry weight	2011	DS	TuDwt11	CE5	SPUD237	10.83	32.2-45.0	38.1
Harvest index(dry weight)		WW	TuDwt11	CE5	Mando	8.38	29.0-43.0	31
CC19DAS	2011	WW	Hldry11	CE5	E45M60-	5	52.4-	19.9
	2010	WW	CC19DAS10	CE1	27h5 PotSNP833	5.77	66.5-52.6-60.3	14.4
		WW	CC19DAS10	CE2	Myb_h17	7.38	91.5-103.8	19.1
		WW	CC19DAS10	CE10	PotSNP111	5.47	22.9-40.4	14
	2011	WW	CC19DAS11	CE2	PotSNP668	7.86	83.3-101.4	18.7
		WW	CC19DAS11	CE10	PotSNP639	6.46	35.0-46.6	14.8

		WW	CC19DAS11	CE11	PotSNP991	6.45	13.6-31.0	14.3
	2011	DS	CC19DAS11	CE10	STM0051	5.38	66.8-73.1	21.2
CC29DAS	2010	WW	CC29DAS10	CE1	Wrky_M3	4.95	45.3-47.2	15
		WW	CC29DAS10	CE2	Myb_h17	5.92	90.6-104.8	20.2
	2011	WW	CC29DAS11	CE1	PotSNP833	5.18	50.6 - 58.3	16.4
		WW	CC29DAS11	CE2	PotSNP108	4.75	79.9-109.3	14.3
CC34DAS	2010	WW	CC34DAS10	CE1	Wrky_H13	5.14	51.6-59.3	15
		WW	CC34DAS10	CE2	Myb_h17	7.94	92.5-103.8	27.7
CC34DAS	2011	DS	CC34DAS11	CE2	PotSNP668	8.98	87.0-100.4	18.4
		DS	CC34DAS11	CE3	PotSNP653	6.81	3.8-12.7	13.9
		DS	CC34DAS11	CE7	PotSNP542	4.94	88.0-107.7	9.3
		DS	CC34DAS11	CE10	PotSNP111	6.31	20-46.0	12
	2011	WW	CC34DAS11	CE2	PotSNP703	4.18	40.8-51.1	16.9

Discussion

Response to drought stress

In the evaluation of 104 individuals of the diploid potato CxE mapping population for morphological, physiological and agronomical parameters for drought tolerance, we observed a significant effect of water shortage on tuber yield production, in agreement with several other drought tolerance studies [3, 13, 18, 19]. All measured growth traits showed a considerable reduction due to water limitation stress, with largest effects on tuber yield and shoot parameters. [7] indicated that the response to drought in potato can be influenced by the severity of water limitation stress and the developmental stage of potato. Since our drought tolerance evaluation was done by completely with-holding water (severe stress) starting from tuber initiation, our discussion will be in view of this scenario. Moreover, how potato responds to water shortage condition can be influenced by the maturity type [13, 20]. In the current study, the positive correlation of maturity with tuber yield indicates that intermediate and late maturing genotypes had better tuber production than early ones under the Ethiopian growth conditions in the field trials. The CxE genotypes have been assessed for developmental traits under short day (Ethiopian) conditions and the life span of the genotypes was shorter compared to the longer life span observed under long day conditions (the Netherlands and Finland) [21]. This indicates that short photoperiod has accelerated the development process through early onset of senescence. However, under both short and long day conditions early genotypes were observed to be early and late genotypes were also observed to be late,

indicating earliness behavior of the genotypes were similar under different day light conditions. Under short day (Ethiopia) conditions genotypes with longer senescence period were reported to have higher tuber yield [21].

Drought stress occurring at the stage of tuber initiation can significantly reduce photosynthesis, biomass production and tuber yield [10]. The strong positive correlation of above ground biomass with tuber yield and tuber dry weight in this study indicates that shoot biomass is an important indicator for yield both under well-watered and drought conditions. [22] indicated that potato genotypes that can maintain above ground biomass under stress conditions are able to produce higher yields. The amount of shoot biomass produced can be influenced by maturity type, as we have observed significant positive correlations with plant maturity under both stressed and non-stressed conditions.

QTLs

Genetically dissecting drought tolerance in the CxE population has resulted in the discovery of 60 QTLs responsible for controlling agro-morphological and physiological traits under drought stress and well-watered conditions. The QTLs discovered under drought stress conditions can be used to enhance potato for tolerance to drought as we define tolerance the ability of genotypes to give higher yield under drought stress condition. As in the other drought studies [13, 16], chromosome 5 had a hot spot region for QTLs identified for several of the traits under drought stress and well-watered conditions. This locus on chromosome 5 was reported to control developmental traits (Plant height, flowering, and senescence) with the CxE population under short photoperiod conditions [23]. In this study, QTLs co-localizing on chromosome 5 were seen in two different positions, from the total 26 QTLs, 25 of the QTLs fell in the interval between 26.2 and 44.0 cM and 1 QTL was located in interval range of 52.4 to 66.5 cM. The latter QTL was identified only for the well-watered treatment. [13] also reported QTLs on these two different positions on chromosome 5, where out of 21 QTLs detected three QTLs found under drought stress conditions were located in the interval range of 47 to 71 cM, while the rest of the QTLs for well-watered, stress and recovery were located between 20 and 48 cM. These results suggest there may be two different, independent QTL present on chromosome 5. For this same potato population, 31 QTLs controlling developmental (plant height, flowering and senescence) and 24 QTLs affecting agronomic traits were reported under short day conditions [23].

Unlike the current study, [13] reported only one QTL on chromosome 5 for well-watered conditions. Although both experiments were done using a similar set of diploid potato genotypes from the same population and similar genetic markers, the difference in the detected QTLs between these two studies could be attributed to the different experimental conditions (field and greenhouse). Besides a genetic component of a trait controlling its expression, environmental factors do have great influence on the expression of quantitative traits [24]. This confirms that selection for drought tolerance is complex. Furthermore, a major QTL for earliness is located on chromosome 5 [25]. The underlying gene of this major QTL was identified as a CDF gene with a pleiotropic effect on many agro-morphological traits of

potato [25, 26] . The CDF alleles controls tuber initiation in potato, and also affects plant maturity. Other studies have identified QTLs on chromosome 5 for maturity, vigour and tuberization [27-29].

The co-localization of QTLs for shoot and root traits with tuber yield on chromosome 5 indicates that the underlying gene(s) have pleiotropic effects (Fig. 2). A QTL for tuber yield was found on chromosome 5 and explained 28.6% of observed phenotypic variation under water stress conditions. Similarly, a QTL for tuber yield on the same locus was reported under stress conditions in a greenhouse experiment [13]. These findings suggest that chromosome 5 harbors important QTL that can be used in marker assisted breeding to improve potato for drought tolerance. QTLs for root length, root fresh and dry weight co-located on chromosome 5 with plant height, shoot fresh and dry weight. The amount of variation explained by these QTLs ranged from 30.9 to 54.2%. Root system architecture is considered important under water stress conditions as it plays a role in avoiding drought stress [4, 24]. Our results indicated that genotypes with strong root expansion had a better tuber yield production under both well-watered and under drought conditions, indicated by the significant positive correlation of root fresh and dry weight with tuber weight. Similarly, other studies have reported positive correlation of root dry mass with tuber yield under water stress [30]. This suggests that root mass may be used as a selection criteria for improving drought tolerance in potato. However, measuring root traits can be difficult and tedious thus focusing on shoot traits that showed high correlation with root traits as an indirect selection criterion for root traits is very practical. Under stress conditions we found that plant height, shoot fresh and dry weight had showed high correlation with root length and root fresh and dry weight. Thus these traits can be used in enhancing potato for tolerance to drought.

A QTL associated with plant height located on chromosome 5 was found under water stress conditions and it had a significant high correlation with tuber weight and tuber dry weight. Although we cannot establish this QTL as drought specific as it was also detected under well-watered conditions, it still can be useful as a selection criterion for higher yields under water-limiting conditions. Similarly, a QTL for plant height was reported on chromosome 5 under stress conditions (that where imposed at the later developmental stage of potato) in a field experiment with the diploid mapping population [16]. This may suggest a locus located on chromosome 5 controls drought responses imposed both at early and late developmental stages of potato. [31] have shown the correlation of plant height with tuber yield under drought stress conditions suggesting this as a morphological trait that can be used as a good indicator of tolerance to drought in potato. Unlike in our study, [13] reported QTL for plant height under stress conditions on chromosome 2 and 7. However, it is important to account for the environmental difference (temperature, relative humidity, soil compositions, etc.) of the experiment that could result in significant differences of QTL detected for similar traits, as the expression of a quantitative trait is not only determined by its genetic component but also by the environment in which it is growing. In our study, the maximum temperature recorded was 29.9⁰C and 29⁰C for the two successive year experiments (Table 3) while [13] reported 33⁰C and 37.9⁰C for their experiment period. Besides, in our experiment other factors such as day length (which was short) and growing medium (which was the field) where quite different compared to [13] experiments where day length was long and a pot was used as growing medium. It has

been reported that shorter day length could restrict plant height growth compared to longer day length under normal field conditions [21]. Therefore, such differences may account for the differences in QTLs expressed under different environmental conditions.

Shoot traits showed high correlation with tuber fresh and dry weight under water stress conditions, which may put them as potential drought tolerance indicator traits that can be used in a potato improvement program. Furthermore, measuring shoot traits is easy, direct, and inexpensive. This may suggest that canopy measurement can be used as selection criteria for genotypes under water limited conditions. The first morphological effect when potatoes are exposed to drought stress is shown in the reduction in leaf size that can affect the canopy architecture [18]. [32] has reported the usefulness of canopy to be used as a selection criterion for nitrogen use efficiency. This study has shown the relationship between canopy development and nitrogen use efficiency. The genomic region controlling shoot fresh and dry weight under stress conditions were found on chromosome 5. Similarly, [13] had reported QTL for shoot fresh weight on chromosome 5 under water stress and recovery conditions. QTLs for harvest index dry, total fresh and dry biomass were also identified on chromosome 5 under drought stress conditions. These findings suggest that the locus on chromosome 5 does not only influence plant maturity but also how genotypes respond to water stress conditions, indicating the effect of maturity on yield under well-watered and drought stress conditions. In our research, late genotypes both under stress and well-watered conditions had higher yield than early maturing ones. This suggests that late maturing genotypes can benefit from longer periods of photosynthesis that would allow production of more assimilates to be partitioned into tubers. However, under drought stress conditions biomass production is tightly linked to transpiration indicating higher yield is associated with increased transpiration efficiency [33]. Therefore breeding for traits such as root dry mass that increases soil moisture capture for transpiration is important.

The relationships that we have seen between tuber yield and physiological and growth traits under drought stress conditions indicate that there is not one trait that can be used as a drought tolerance indicator; rather it is the aggregated effect of different traits. However, there is a difference in the amount of variation explained by the QTLs linked to these different traits. Moreover, the heritability of a trait is important to consider since response to selection for drought is efficient if traits show correlation to yield and have a high heritability [34, 35]. Heritability in the current study ranged from moderate to high for several of the traits measured under drought stress conditions. Heritability estimates provide a basis for predicting the response to selection in drought tolerance improvement programs. The higher the heritability estimate, the better the response to selection. Similar heritability ranges for drought tolerance experiments were reported by [13, 16]. Since direct selection for tuber yield under drought stress conditions is difficult in most cases, agro-morphological, and physiological traits that have correlation with tuber yield and higher heritability can be used as drought tolerance indicators for an indirect selection.

Drought specific QTLs were detected for tuber yield on chromosome 7, 9, and 12. Also, on chromosome 8 we have found a QTL for plant height specific to stress conditions. These QTLs were expressed only in

one experimental year (Table 2; Fig. 2). This implies that the expression of the QTLs may be influenced by the difference in environmental conditions (temperature, relative humidity, etc.) between different years (Table 3). The main difference between the two experimental years was the minimum temperature recorded, 10.8°C in year 2010 and 4.5°C in 2011. The stability of these QTLs being expressed under water stress conditions might need to be confirmed in a further field trial. However QTL detected on chromosome 12 for tuber fresh weight under drought stress conditions showed an overlap with QTL detected for onset and inflection point of plant height under normal growing conditions in [23]. Also, QTL identified on chromosome 8 for plant height specific to drought stress condition co-located with QTL identified (single trait QTL analysis) on chromosome 8 for a parameter controlling inflection point of senescence under short photoperiod conditions [23]. The QTL detected on chromosome 7 for tuber yield under water stress conditions co-located with a QTL found for tuber number under well-watered conditions indicating the same locus controls tuber number under both treatment conditions. QTL for tuber number under well-watered conditions was also detected on chromosome 3. Similarly, QTL for tuber number under short photoperiod conditions was reported at the same chromosome in [23].

A QTL for harvest index based on dry weight was detected on chromosome 5 only under well-watered conditions, [16] reported QTLs for harvest index based on fresh and dry weight on chromosome 5 for water stress and well-watered conditions. Their harvest index data from fresh weight was collected from both green house and field trials, while data of harvest index from dry weight was collected from green house only. On chromosome 8 a QTL for root dry weight co-located with tuber number and tuber yield under well-watered conditions. Chromosome 8 was reported to contain QTLs for tuber fresh weight, tuber dry weight, harvest index fresh weight under well-watered conditions of a greenhouse experiment [16]. However, we were not able to compare the co-localization of the QTLs on the same position since different genotypes of the population and markers were used. Nevertheless, this finding indicates that QTL located on chromosome 8 is expressed under different environments (greenhouse and field) and different mapping population. For two of the experimental years QTL for plant height measured under well-watered conditions was detected on chromosome 6, indicating the stability of the QTL being expressed in different years.

Chlorophyll content (SPAD measurement) was indicated to have a close correlation with leaf photosynthetic capacity [36, 37]. Stay green traits are related with maintaining higher chlorophyll content under drought stress conditions and this is associated with higher photosynthetic capacity and better yield [38] In Sorghum, stay green traits are associated with delayed senescence resulting in better yield and biomass under drought stress conditions [39]. Therefore maintaining higher chlorophyll content under water stress conditions can help a plant to cope with the effect of stress. In the current study, we have detected 7 QTLs for chlorophyll content on chromosome 2 that were expressed at different time points, 19, 29 and 34 DAS, and in both years, under both drought stress and well-watered conditions. Similarly, [13] identified a QTL on chromosome 2 for chlorophyll content measured 3 DAS under water stress conditions that overlaps with the QTL positions detected in the current study. This suggests that the QTL present on chromosome 2 for chlorophyll content is a stable QTL that is expressed across

treatments, years and environments. Chlorophyll content measured under drought conditions was higher than under well-watered conditions in all the three time points measured (Table 1) and this may suggest a possible reduction in leaf size making the leaf appear darker. Increased leaf greenness was reported as a consequence of reduced leaf growth in potato rather than as a delayed senescence under moderate drought stress imposed at onset of tuber initiation [40]. However, other researchers suggest leaf greenness under drought stress conditions can be associated with delayed senescence in potato under drought stress conditions [41, 42]. The different explanation given by these authors about increased chlorophyll content in response to water stress might be linked to the different time and level of stress applied. Therefore, it is important to measure leaf area or score senescence under drought stress conditions in order to confirm chlorophyll content as drought tolerance indicators. In addition, it was reported to consider the developmental stage in which chlorophyll content is measured and the timing and level of stress imposed in order to consider increased leaf greenness as an indicator of delayed senescence [40]. Four other QTLs for chlorophyll content were identified on chromosome 10 for chlorophyll content measured on 19 and 34DAS for both treatment conditions. Chromosome 10 was also reported to contain QTL for chlorophyll content measured on 3 and 8DAS under stress conditions of a greenhouse experiment [13], moreover this QTL was reported to co-locate with an eQTL belonging to the functional classes of global transcription factors group and putative DNA-binding protein [13]. Under well-watered conditions, QTL for chlorophyll content co-located with shoot fresh weight and total fresh biomass on chromosome 11, suggesting a relationship between chlorophyll content and growth traits however the correlation observed between these traits is weak. QTL for chlorophyll content were detected on chromosome 1 under well-watered conditions however it did not co-locate with QTL identified for root dry weight under well-watered conditions.

Conclusions

All these QTLs found under drought stress conditions indicate that there is genetic variation in this population that can be used in the improvement program of potato for drought tolerance. Furthermore, studies like these give a better insight in drought tolerance traits that can be used to enhance potato for drought tolerance. However, it is important to confirm stability of drought related QTLs in order to be implemented in marker assisted breeding program. Finding stable QTL for drought tolerance traits, that are expressed across environments can be complex since response to drought is highly influenced by the different developmental stage of potato in which stress is being applied as well as by the severity level of stress imposed. Accounting for different stress scenarios and developmental stages of potato in drought stress experiments could help in finding stable QTLs for drought tolerance traits that can be used to implement in marker assisted selection of potato for enhanced drought tolerance.

Methods

In two consecutive years (2010: Experiment 1 and 2011: Experiment 2), a field experiment was carried out in a semi-arid environment at Melkassa, Ethiopia. The area is characterized by an average day

temperature of 28⁰C, annual rainfall of 928 mm and is situated at 1550 meters above sea level (masl) at coordinates 8⁰24'N 39⁰21'E, with clay loam soil. The weather characteristics during the experiment period is given in table 3.

Plant materials

A set of 104 diploid potato mapping population from crosses of two diploid potato clones, USW53373.3 coded C and 77.2102.37 coded E was used. Clone C is a hybrid between *S. phureja* PI 225696.1 and the *S. tuberosum* dihaploid USW42. Clone E is a cross between clone C and the *S. vernei* – *S. tuberosum* backcross clone VH³4211. The full description of the population can be obtained from [43]. The selected population largely overlaps with the set used by [13].

Experimental design

A split plot design with two blocks was used and the 104 CxE diploid potato genotypes together with Parents C and E were randomized within a block. The water regime was allocated to the main plot and genotypes were assigned to subplots with two replications. In each replication eight plants per genotype were planted and planting was done manually. The distance between and within row were 75 cm and 30 cm, respectively. A split application of Urea at planting and flowering was done at a rate of 165kg/ha and Di-ammononium phosphate (DAP) was applied at planting at a rate of 195kg/ha. Furrow irrigation was used to water the field with an average interval of four days. The four replicates were kept watered until tuber initiation, watering was ceased for two of the replications at the time of tuber initiation and the plants were without irrigation for 38 days in the first experiment and 50 days for the second experiment. For both years harvesting was done at the end of the drought stress period (73 and 75 days after planting for the first and second experiment, respectively).

All the field cultivation practices were done manually. The field was kept free of weeds by hand weeding whenever necessary. During the growing periods ridging of the field was done four times. The field was sprayed with Redomil Gold and Mancozeb to control late blight (*Phytophthora infestance*) infestations and applications were done six times. The chemical Selecron was sprayed against cut worm (*Agrotis ipsilon*), Agro-methiotate against potato tuber moth (*Phthorimaea operculella*), and Mancozeb against potato stem blight (*Sclerotium rolfsii*). All the diseases were controlled except for late blight disease. Late blight infection was scored using a disease scoring scale of the Centro Internacional de la Papa (CIP) technical bulletin JW Henfling [44].

Table 3 Weather data collected during the field experiment at Melkassa

Year	Parameters	September	October	November
2010	Minimum Temperature (°C)	15.2	11	10.8
	Maximum Temperature (°C)	27.6	29.9	28.7
	Relative humidity (%)	68	46	50
2011	Minimum Temperature (°C)	8.8	4.5	6.7
	Maximum Temperature (°C)	26.7	29	28
	Relative humidity (%)	70.7	41	53

Phenotyping

Data collection started on average 16 and 12 days after planting (DAP) by scoring emergence in 2010 and 2011 respectively. Emergence scoring was continued with daily observations until all eight plants of each of the 104 genotypes and 17 cultivars had emerged. For the two experimental years, number of main stems was counted and plant height was measured from the tip of the plant to the soil level by selecting the longest main stem.

In the 2011 experiment, for shoot and root biomass measurements, shoots and roots were weighed immediately after harvest as fresh weight and after oven drying at 105°C for 48 hours as dry weight. For measuring root length, each side of the plant was carefully dug out to uproot the plant and roots were cleaned with water. The longest root length was then measured with a ruler. Tubers larger than 20mm in diameter were counted and weighed as tuber number and yield. For tuber dry weight measurement, tubers were sliced and dried in an oven at 80°C for 72 hours. Tuber yield data is available for both experimental years. Total fresh biomass (g), was calculated by adding fresh weight of shoot and tuber. Similarly, total dry biomass (g) was calculated. Harvest index based on dry weight was calculated by dividing tuber dry weight by total dry biomass. Root to shoot ratio (R:S) was calculated as the ratio of root dry weight and shoot dry weight.

For both experimental years, Chlorophyll content was measured at 19, 29 and 34 DAS (CC19DAS, CC29DAS and CC34DAS) with a SPAD 502 chlorophyll meter (Minolta Co., Japan). Measurements were done on the third leaflet from the top young fully expanded leaflet.

Despite the use of different fungicide chemicals, late blight disease could not be fully controlled. Drought-stressed plants were more affected than well-watered plants. The incidence level of the disease was scored on a scale from 1 – 9 following CIP guideline JW Henfling [44]. Disease score was done at different time points; 39 days after stress (DAS) for 2010 and 22, 33 and 44 days after stress for 2011. In order to account for the effect of disease effects possibly confounding the effects of the drought stress on the phenotypic traits, disease score (39 DAS for 2010 and 44 DAS for 2011) was used as a covariate in the analysis of variance.

Statistical analysis

All the data collected for both treatments were statistically analyzed using Genstat 15th edition [45]. For correcting drought tolerance traits for disease effect, the disease incidence scored at 44DAS was used as a covariate in analysis of variance (ANCOVA), since it coincides with the timing of drought trait data collected at the end of stress. Broad sense heritability (H^2) was computed as $H^2 = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_e}$, where σ^2_g is genetic variance, σ^2_e is environmental variance, and n is number of replications. Relative reduction of traits was calculated as a difference between control and drought which then divided by the control mean and expressed in terms of percentage.

Genetic map construction

The marker data used to construct the genetic map used in this study is described in [13]. The marker data includes Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), Cleavage Amplified Polymorphism (CAP) and Single Nucleotide Polymorphism (SNPs). The genetic map order and positions developed by [13] were used to reconstruct a genetic map for the CxE progeny used in this study. Joinmap 4.1 [46] was used to construct an integrated CxE genetic map. We have used 529 markers in total and markers with LOD value above 5 were included for map construction.

QTL analysis

QTL analysis was done separately for control and drought stress treatments using MapQTL 6 [47]. Interval mapping was done first to identify and locate QTL on the linkage group. A permutation test was performed to determine significant QTL and a genome wide LOD threshold level of $P < 0.05$ was used to declare presence of significant QTLs. Following this, restricted multiple QTL mapping (rMQM) was done and markers for QTLs detected by interval mapping were used as a co-factor. This procedure was continued until a stable list of cofactors was obtained. The integrated maps and QTLs were drawn using MapChart 2.2 [1] .

Declarations

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Authors' contributions

BBT designed and carried out the field experiments, performed the analysis and prepared the manuscript. GG involved in planning field experiments. RV and GL involved in conceptualizing the project, participated in interpretation of results and revision of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Pearson correlation showing relationships of traits measured at the end of drought stress period under drought stress (lower triangle) and well-watered (upper triangle) conditions. The diagonal indicates the correlation between the two different environments PM= plant maturity, StNr= stem number, PIHt= plant height, SFW= shoot fresh weight, SDW= shoot dry weight, RFW= root fresh weight, RDW= root dry weight, RL= root length, R:Sh= root to shoot ratio (dry weight), TFB= total fresh biomass, TDB= total dry biomass, TuNr= tuber number, TuFWt= tuber fresh weight, TuDWt= tuber dry weight, HidryW= harvest index dry weight, and CC19DAS, CC29DAS and CC34DAS is chlorophyll content measured on 19, 29, and 34 days after stress.

Figure 2

CE integrated linkage map with QTL detected under drought stress (red bar) and well-watered (green bar) conditions. Markers names are shown on the right side of the linkage group, with their positions indicated on the left. Bars indicate QTL, with the 2 LOD (Solid fill) and 1 LOD (dotted line) support interval indicated as well. Trait names and experimental year located on QTL bars, for trait names refer table 2.