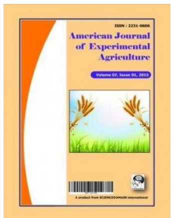

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A Rapid Morphological Screening Procedure for Pea (*Pisum sativum* L.) under Drought Stress in Greenhouse Settings

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ETB and BWB designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors KB and AN reviewed the experimental design and all drafts of the manuscript. Author ETB managed the analyses of the study. Author BWB identified the plants. Author ETB performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This work proposes a rapid morphological screening procedure for peas grown under drought stress aiming to provide phenotypic evaluations for further research.

Materials and Methods: The plant yield and morphological changes of two widely grown pea cultivars ('Aragorn' and 'Banner') to drought stress was evaluated in this work. A total of eighty seeds of each pea variety were grown in a randomized completed block with four replicates in a greenhouse located in Moscow, ID. Plants were subjected to optimal irrigation (1400 ml as a control) and two treatments that reduced optimal irrigation rates by 40% (840 ml, moderate drought) and

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60% (560 ml, severe drought) to induce drought stress. The test was repeated.
Results: Varieties significantly (P<0.05) differed in their response to water deficiency. The variety 'Banner' appeared to be the most drought tolerant than 'Aragorn' with high values at control, moderate and severe as (85.00±4.08) cm, (87.21±3.26) cm and (66.02±2.92) cm respectively for total plant height, while the values for total dry weight were (3.65±0.20) g, (2.18±0.42) g and (1.26±0.10) g respectively. Similarly, there were significant (P<0.001) differences among treatments on growth parameters, with highest values recorded at optimal irrigation (1400 ml) as (21.52±2.42) cm, (62.18±4.68) cm, (0.24±0.04) g, (2.86±0.38) g and (2.30±0.18) seeds/pod for root length, shoot height, root weight, shoot weight and number of seeds respectively. The growth parameters decreased with a decrease of irrigation.
Conclusion: As drought and stress conditions are expected to increase as global climate change progresses, breeding for drought is a promising area. Therefore, the variety 'Banner' has been identified as a potential parental material to be used in breeding for drought tolerance.

Keywords: Drought; greenhouse plants; agronomic methods; stress tolerance; peas.

1. INTRODUCTION

Over the past several decades, drought stress has become one of the biggest concerns in agriculture worldwide. Adaptation to abiotic stresses is an important requirement of agriculturally relevant crops and development of enhanced drought tolerance plays an essential role in plant breeding [1].

The development of drought tolerant plants is of increasing priority and relevance as the area affected by significant drought is expected to markedly increase in the future due to global climate change [2]. While progressive water withdrawal has been studied in the greenhouse for beans, it generally does not mimic actual field conditions [3].

While limited studies and techniques for simulating field drought stress in a greenhouse environment have been conducted [4], more studies are needed to adequately formulate a reliable protocol for legumes, especially peas. It is the aim of this research to provide a rapid morphological screening procedure for pea under drought stress in greenhouse settings.

The use of peas as a model crop for genetic studies has a very rich history, beginning with Mendel in the 1860s. *Pisum sativum* is adapted to cool, semi-arid to sub-humid growing conditions [5]. However, pea has been grown in diverse environments across the globe and the demand for production continues to increase [6]. Drought is of great concern in pea and it has been reported to result in a loss of between 38.5 and 43% production under different stress levels [7].

2. MATERIALS AND METHODS

Two dry pea cultivars, 'Aragorn' and 'Banner,' commonly grown in the U.S. and New Zealand and developed by ProGene Plant Research LLC in conjunction with NZ Plant Research LTD were utilized for this experiment. 80 seeds of each genotype were prepared by sterilization utilizing a 10% bleach aqueous solution, rinsed in sterile, distilled H_2O and air dried for a period of 24 hours.

Five seeds of each variety were planted in a 3.75 liter high density polyethylene/polypropylene polycan container (Anderson Pots, Oregon, USA) filled with 690 g of Sunshine Mix #1 growing media (Sun Gro Horticulture, Agawam, MA) and arranged in a complete randomized design with four replicates per cultivar. The experiment was replicated in triplicate.

The peas were grown under controlled greenhouse conditions with temperatures ranging from 12 to 18ºC at night and 21 to 27ºC during daylight hours (Micro Grow Greenouse Systems Growmate (Temecula, CA) utilizing 400 Philips HID Ceramalux High Pressure Sodium bulbs). The plants were subjected to a 12-hour photoperiod per 24 hours.

Three different irrigation regimes were tested: control, moderate drought and severe drought. Field capacity of the growing media served as the experimental control. Field capacity was determined by wetting 690 g of media until saturation was reached. The media was then allowed to drain for a period of 24 hours and oven dried to determine maximum moisture holding capacity (field capacity).

Previous field trials conducted by Pro Gene Plant Research [8] have shown that approximate reductions in irrigation of 40% and 60% caused visual drought stress symptoms on plots of field peas. Control plants were irrigated to the full field capacity of the growing media (1400 ml) and irrigated once every 7 days until flowering and then once every 3.5 days from flowering until maturity. Drought stress test pots received amounts of water decreased by 40% (840 mL) and 60% (560 mL) of field capacity, respectively, at the same watering interval.

Phenotyping methods consisted of the collection of the following agronomic notes: emergence and flowering dates, plant survival, shoot height and root length, shoot and root dry weight, number of pods per plant, pod length and width, and number of viable seeds produced per plant. Statistical analysis of the data, including the Fisher's LSD method, was performed using Minitab 17 Statistical Software (Minitab, State College, PA) and Agrobase Generation II® (Agronomix Software Inc., Winnipeg, Manitoba).

3. RESULTS AND DISCUSSION

As shown in previous research, emergence and flowering dates were not affected by drought stress treatments [9]. All 'Banner' treatments (including control) flowered 52 days after planting, while all 'Aragorn' treatments (including control) flowered 54 days after planting. However, varieties differed in their response to water deficiency and plant survival was jeopardized. The 40% reduction from field capacity trial yielded a 20% rate of mortality for both varieties tested. The 60% reduction from field capacity trial resulted in 20% mortality in 'Aragorn' and 27% in 'Banner.' For all morphological measurements: shoot height, shoot dry weight and number of seeds per pod showed a consistent decrease due to drought for both varieties (Table 1).

A statistically significant ($P<0.001$) difference in shoot height was observed in both variety and treatment ($P<0.001$) comparisons. 'Banner' was taller than 'Aragorn' in all treatments and both varieties suffered from increasing stunt with relation to decreasing irrigation (Fig. 1).

'Banner' and 'Aragorn' exhibited a 6.3% and 9.8% reduction in plant shoot height, respectively, in the moderate drought treatment. Both had the least shoot height, at only 70% of their potential, when compared to the control plants, in the severe drought treatment. Shoot dry weight was also impacted by drought, being significantly different ($P<0.001$) among treatment and between varieties. For the moderate and severe drought stress treatments, 'Banner' shoot dry weight (Fig. 2) was reduced by 44% and 70%, respectively, while 'Aragorn' was reduced by 55% and 69%, respectively.

Growth reduction was one of the major drought stress treatments results. It was also observed that leaf area was visually reduced. In a similar study involving wheat cultivars, leaf area was also reduced but root development was retarded in only one cultivar [10]. A reduction in plant height, lower leaf and stem dry weights were also shown, in addition to the production of a lower overall aerial biomass among drought-stressed plants grown in a greenhouse environment [4].

The maintenance of root growth as observed in 'Banner' under drought stress condition can be an important character of drought tolerant plants. Root length did not significantly $P>0.05$) differ among treatments and between varieties. 'Aragorn' roots were not consistently affected on the moderate drought treatment but had an important reduction of 32% on root length on the severe drought; while 'Banner' roots were not consistently affected between the treatments. Root dry weight did not differ ($P>0.05$) significantly among treatments and between varieties. Overall, 'Aragorn' produced more roots than 'Banner' in all trials.

The number of pods per plant were highly significantly ($P<0.001$) different among treatments and also showed a significant ($P<0.05$) difference between varieties. In all treatments, 'Banner' had more pods than 'Aragorn' but both varieties exhibited a reduction in the number of pods with increasing drought. 'Banner' displayed a 38.5% and 41.5% reduction in the overall number of pods as drought increased, respectively; while 'Aragorn' had a reduction of 42.7% and 56%, respectively.

In addition, pods also became shorter and thinner with decreasing irrigation when compared to the control (Table 1). 'Aragorn' did not produce any viable seeds in either drought treatment while 'Banner' was able to produce viable seed throughout all treatments. 'Banner' suffered a reduction in the number of viable seeds by 19% in the moderate drought treatment and 27% in the severe drought treatment.

Yield measured by yield components (number of pods, pod length, width, and number of viable seeds per pod) was also reduced under simulated drought conditions, with simulated severe drought underperforming all other treatments. In a similar study, analysis of variance has indicated that there were significant differences between genotypes regarding agronomic and morphological traits of wheat under drought [11].

When both varieties are combined for the analysis in each treatment it is possible to observe the overall effect of drought on the investigated plant material. Significant differences can be then observed across treatments for all the evaluated parameters (Table 2).

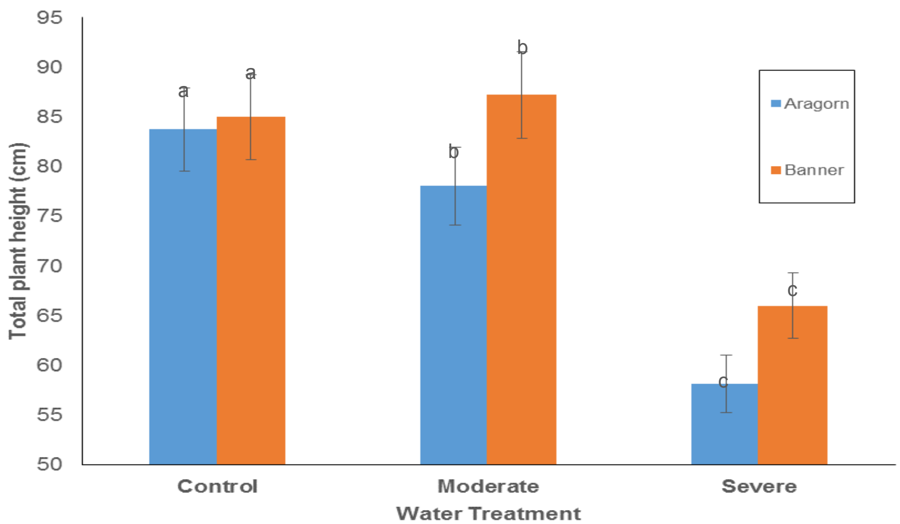

Fig. 1. Plant total height (cm) of 'Banner' and 'Aragorn' across water treatments - control (1400 ml), moderate drought (840 ml) and severe drought (560 ml) stress. Total height included shoot and root measurements. The letters a, b, and c represent the grouping information for the treatments using Fisher LSD method and 95% confidence. Means that do not share a letter are significantly (P<0.05) different

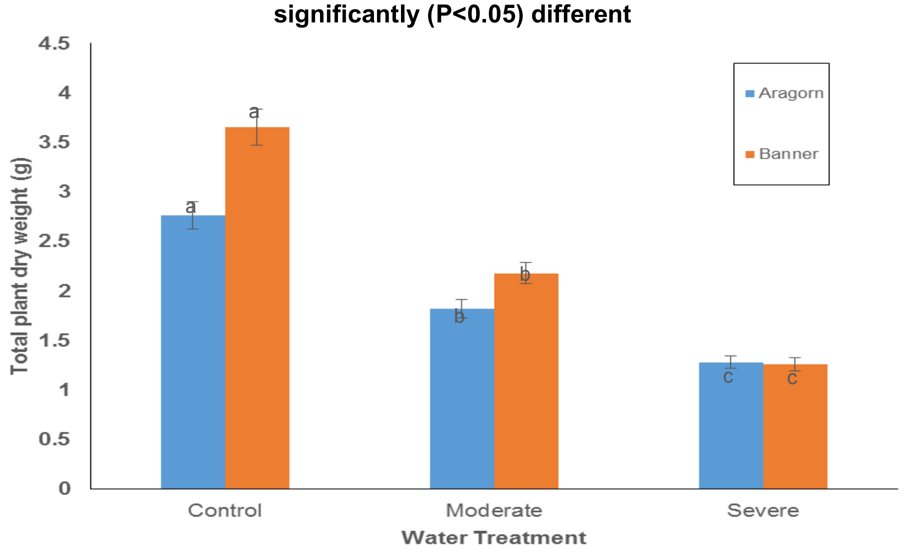

Fig. 2. Total plant dry weight (g) of 'Banner' and 'Aragorn' across water treatments: control (1400 ml), moderate drought (840 ml) and severe drought (560 ml) stress. Total dry weight included shoot and root measurements. The letters a, b, and c represent the grouping information using Fisher LSD method and 95% confidence for the treatments. Means that do not share a letter are significantly (P<0.05) different

Table 1. Morphological data means collected in each treatment (control, moderate, and severe drought), showing: varieties, root length, shoot height, shoot weight, root weight, pod length, pod width, number of pods per seed, number of seeds per pod, and mortality

Irrigation treatment	Variety name	Root length (cm)	Shoot height (cm)	Root weight (g)	Shoot weight (g)	Pod length (cm)	Pod width (cm)	Number of pods/ plant	Number of seeds/pod	Mortality %
Control	Aragorn	22.54±2.1	61.21±2.6	0.32±0.04	2.44±0.2	2.99±O.67	0.29±0.07	2±0.22	0.60±0.4	0
	Banner	17.57±1.2	67.43±3.9	0.18±0.02	3.47±0.33	4.79±0.24	0.59±0.01	2.6±0.23	2.75±0.45	0
Moderate	Aragorn	22.79±2.58	55.27±1.6	0.27±0.03	1.55±0.14	1.55±0.03	0.067±0.01	1.08±0.23	0.00	20
	Banner	24.02±2.56	63.19±2.03	0.23±0.03	1.95±0.13	4.22±0.3.2	0.55±0.04	1.5±0.23	2.23±0.37	20
Severe	Aragorn	15.25±1.5	42.93±2.03	0.19±0.031	1.09±0.13	1.91±0.46	0.12±0.05	0.83±0.27	0.00	26
	Banner	19.48±1.48	46.54±2.52	0.19±0.02	1.07±0.1	3.92±0.39	0.59±0.02	1.07±0.19	2.00±0.33	27

Table 2. Morphological data means across treatments (control, moderate and severe drought) combining both varieties

Treatment/parameters	Control*		Moderate*		Severe*	
Root length	21.52±2.42	A	19.57±3.65	A	15.57±2.11	B
Shoot height	62.18±4.68	A	54.37±2.56	B	40.22±3.20	C
Root weight	0.24±0.04	A	0.23±0.04	AB	0.17±0.04	B
Shoot weight	2.86±0.38	A	1.58±0.19	B	0.96±0.13	C
Number of pods	2.30±0.18	A	1.29±.17	B	0.92±16	C

** Grouping information using Fisher LSD method and 95% confidence. Means that do not share a letter within a row are significantly (P<0.05) different*

Our data suggests that plant characteristics can influence drought tolerance. Comparing the two varieties used in our study, 'Banner,' the variety that performed better under drought stress, has good early vigor emerging five to six days earlier than 'Aragorn' [8].

There are many metabolic changes that can enable a plant to withstand drought, including specific proteins such as chaperonins and dehydrins that are induced in certain plants under drought [12]. Water stress also induced the accumulation of soluble sugars in epicotyls between 2.8- and 5.1-fold in another study and the osmotic adjustment varied greatly depending on cultivar [13]. Moreover, temperature can contribute to drought stress and can induce significant changes in plant cell gene expression [14].

4. CONCLUSION

Our study has proposed a simple, rapid morphological screening procedure for pea under drought stress in greenhouse settings and provided evaluation of two commercial pea cultivars. 'Banner' appeared to be the most drought tolerant, which was reflected by both growth and yield parameters. 'Aragorn' had shown many drought stress symptoms even in the moderate drought treatment, such as very low yield. Our work also identified potential parental material to be used in breeding. As drought and stress conditions are only expected to increase as global climate change progresses, breeding for drought is a promising area.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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