

Short Research Article

A Rapid Morphological Screening Procedure for Pea (*Pisum spp.*) Under Drought Stress in Greenhouse Settings

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

Material 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 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.

Conclusions: 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

26 reliable protocol for legumes, especially peas. It is the aim of this research to provide a rapid
27 morphological screening procedure for pea under drought stress in greenhouse settings.

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

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35 2. MATERIAL AND METHODS

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37 Two dry pea cultivars, 'Aragorn' and 'Banner,' commonly grown in the U.S. and New
38 Zealand and developed by ProGene Plant Research LLC in conjunction with NZ Plant
39 Research LTD were utilized for this experiment. 80 seeds of each genotype were prepared
40 by sterilization utilizing a 10% bleach aqueous solution, rinsed in sterile, distilled H₂O and air
41 dried for a period of 24 hours.

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

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

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

56 Previous field trials conducted by ProGene Plant Research [8] have shown that approximate
57 reductions in irrigation of 40% and 60% caused visual drought stress symptoms on plots of
58 field peas. Control plants were irrigated to the full field capacity of the growing media (1400
59 ml) and irrigated once every 7 days until flowering and then once every 3.5 days from
60 flowering until maturity. Drought stress test pots received amounts of water decreased by
61 40% (840 mL) and 60% (560 mL) of field capacity, respectively, at the same watering
62 interval.

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

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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).

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±0.67	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

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 (Figure 1).

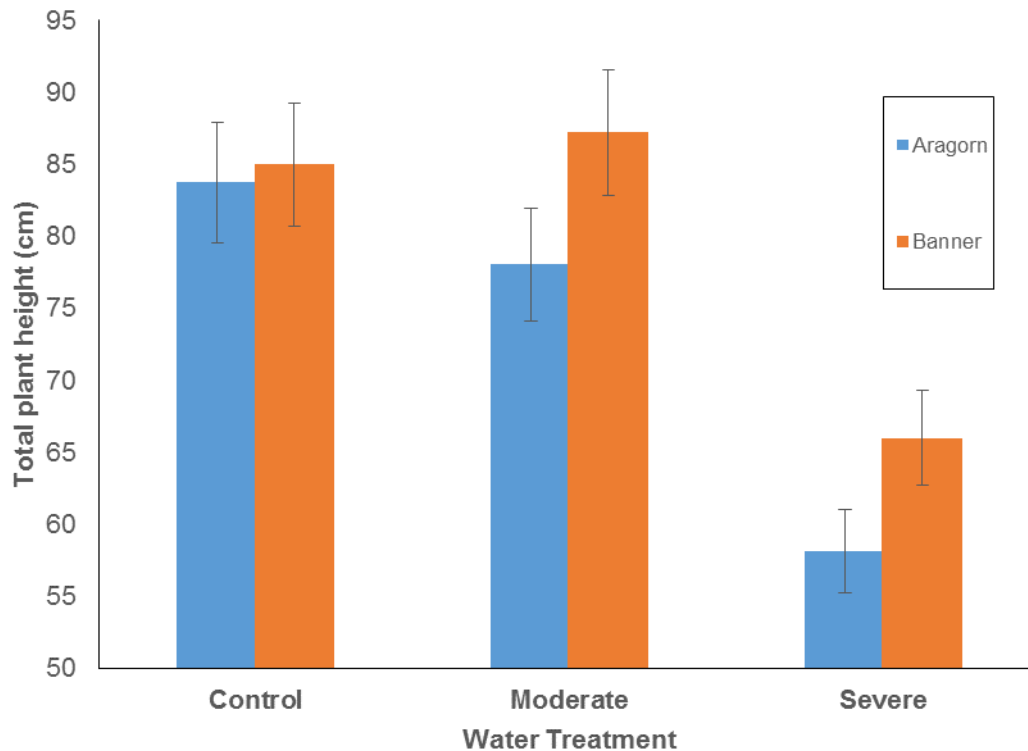
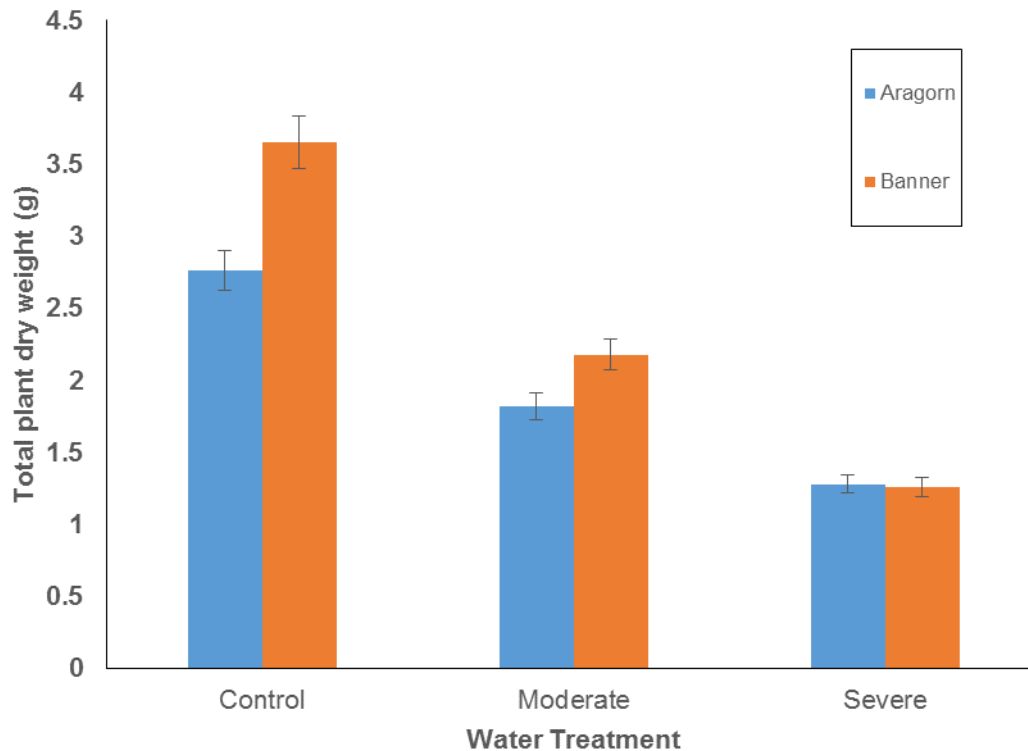


Figure 1 Plant total height (cm) of 'Banner' and 'Aragorn' across water treatments - control (1400 ml), moderate drought (840 ml) and severe drought (560 ml) stress. Total height included shoot and root measurements.

'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 (Figure 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].



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113 Figure 2 Total plant dry weight (g) of 'Banner' and 'Aragorn' across water treatments: control
 114 (1400 ml), moderate drought (840 ml) and severe drought (560 ml) stress. Total dry weight
 115 included shoot and root measurements.

116 The maintenance of root growth as observed in 'Banner' under drought stress condition can
 117 be an important character of drought tolerant plants. Root length did not significantly ($P>0.05$)
 118 differ among treatments and between varieties. 'Aragorn' roots were not consistently
 119 affected on the moderate drought treatment but had an important reduction of 32% on root
 120 length on the severe drought; while 'Banner' roots were not consistently affected between
 121 the treatments. Root dry weight did not differ ($P>0.05$) significantly among treatments and
 122 between varieties. Overall, 'Aragorn' produced more roots than 'Banner' in all trials.
 123 The number of pods per plant were highly significantly ($P<0.001$) different among treatments
 124 and also showed a significant ($P<0.05$) difference between varieties. In all treatments,
 125 'Banner' had more pods than 'Aragorn' but both varieties exhibited a reduction in the number
 126 of pods with increasing drought. 'Banner' displayed a 38.5% and 41.5% reduction in the
 127 overall number of pods as drought increased, respectively; while 'Aragorn' had a reduction of
 128 42.7% and 56%, respectively.

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

134 Yield measured by yield components (number of pods, pod length, width, and number of
 135 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).

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 ± 0.17	B	0.92 ± 0.16	C

* Grouping information using Fisher LSD method and 95% confidence. Means that do not share a letter within a row are significantly (P<0.01) 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.

168 **COMPETING INTERESTS**

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170 Authors have declared that no competing interests exist.

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173 **REFERENCES**

174

175 1. Witt S, Galicia L, Lisec J, Cairns J, Tiessen A, Araus JL, Fernie AR. Metabolic and
176 phenotypic responses of greenhouse-grown maize hybrids to experimentally controlled
177 drought stress. *Molecular plant*. 2011; ssr102.

178 2. de Paiva Rolla AA, Carvalho JDFC, Fuganti-Pagliarini R, Engels C, do Rio A, Marin SRR,
179 Oliveira MCN, Beneventi MA, Corrêa F, Guimarães M, Farias JBC, Neumaier N,
180 Nakashima K, Yamaguchi-Shinozaki K, Nepomuceno AL. Phenotyping soybean plants
181 transformed with rd29A: AtDREB1A for drought tolerance in the greenhouse and field.
182 *Transgenic research*. 2014; 23(1), 75-87.

183 3. Kavar T, Maras M, Kidric M, Sustar-Vozlic J, Meglic V. Identification of genes involved in
184 the response of leaves of *Phaseolus vulgaris* to drought stress. *Mol Breeding*. 2008; 1(21),
185 159-172.

186 4. Pennypacker BW, Leath KT, Stout WL, Hill RR. Technique for simulating field drought
187 stress in the greenhouse. *Agronomy journal*. 1990; 82(5), 951-957.

188 5. Coyne C J, McGee RJ, Redden RJ, Ambrose MJ, Furman BJ, Miles CA. Genetic
189 Adjustment to Changing Climates: Pea (pp. 238-250). Wiley Blackwell: Chichester, UK.
190 2011.

191 6. EMBRAPA – Empresa Brasileira de Pesquisa Agropecuária. Centro Nacional de
192 Pesquisa de Hortaliças. Ervilha para produção de grãos verdes. 2 Nov. 2008.
193 <www.cnph.embrapa.br/cultivares/ervilha>.

194 7. Carvalho JA, Rezende, FC, Aquino RF, Freitas WA, Oliveira EC. Produção da ervilha
195 cultivada em ambiente protegido sob diferentes tensões de água no solo. *Revista Brasileira*
196 *de Engenharia Agrícola e Ambiental*, Campina Grande. 2012; 1(16), 44-50.

197 8. Braunwart K, Wood M. Drought Stress on Pea Crops. *ProGene Plant Research*; 2014.

198 9. Muchow RC. Phenology, Seed yield and WaterUse of Grain Legumes Grown under
199 Different Water Regimes in a Semi-arid Tropical Environment. *FieldCrops Research*. 1985;
200 11, 81-87

201 10. Klamkowski K, Treder ww. Response to Drought Stress of Three Strawberry Cultivars
202 Grown Under Greenhouse Conditions. *J. Fruit Ornament. Plant Res*. 2008; 180 (16):179-188.

203 11. Ahmadizadeh M, Nori A, Shahbazi H, Aharizad S. Correlated response of morpho-
204 physiological traits of grain yield in durum wheat under normal irrigation and drought stress
205 conditions in greenhouse. *African Journal of Biotechnology*. 2014; 10(85), 19771-19779.

206 12. Vaseca I, Akiscan Y, Demirevska K, Anders I, Feller U. Drought stress tolerance of red
207 and white clover: comparative analysis of some chaperonins and dehydrins. *Scientia*
208 *Horticulturae*, Canada. 2011; 4(130), 653-659.

- 209 13. Sánchez FJ, Andrés EF, Tenorio J, Ayerbe L. Growth of epicotyls, turgor maintenance
210 and osmotic adjustment in pea plants (*Pisum sativum* L.) subjected to water stress. *Field*
211 *Crops Research*. 2014; 2(86), 81-90.
- 212 14. Talalaiev O, Korduym E. Expression of small heat shock protein (sHSP) genes in the
213 garden pea (*Pisum sativum*) under slow horizontal clinorotation. *Plant signaling & behavior*.
214 2014; 9(4), e29035.