

The effect of pH on roots, shoots, leaves and germination rate during the early developmental stage of cowpea (*Vigna unguiculata* L.)

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Abstract

Cowpea is a rich and cheap source of protein for both human and animal consumption. However, cowpea production is on a decline due to current environmental factors such as soil acidity, drought, and heat. This experiment was carried out to observe how five different pH levels (4, 5, 6, 7 and 9) could affect the germination rate and to analyze the relationship between root growth and shoot and leaf development. For each pH level, 25 seed samples were used to determine the germination rate. At 24hrs, there was little variation of germinated seeds based on the different pH, while at 72hrs, all seed samples regardless of the pH level attained a 100% germination rate. Three out of the 25 germinated seeds were randomly selected and grown in a liquid media under a controlled environment for three weeks. Fresh and dry weight of leaves, shoots and roots were measured. Pearson's correlation showed a strong negative relationship between the number of leaves and root length with high significance ($P < 0.01$), this negative correlation could be important in pasture design for animal husbandry as vegetative cover takes greater precedence over root growth. Although, the number of leaves was the same for pH7 and pH9, however, the latter had the lowest leaf mass while the former had the highest, the difference in their masses could be because of low water flow and hydraulic conductivity at lower pH. Also, other correlations were observed between traits such as masses of shoots, roots, leaves.

Keywords: *pH, germination rate, root, shoot, leaves*

1. Introduction

Cowpea (*Vigna unguiculata*) is an important legume in sub-Saharan Africa due to its high tolerance against drought and other environmental factors (Boukar et al., 2013). Cowpea is an annual crop and a member of the Fabaceae family. In developing countries, cowpea is grown in mixed cropping system with stands of cereals such as maize, millets and sorghum (FAO, 2013). The western part of Africa contributes around 95% of the global production of cowpea (Boukar et al., 2013), this high production highlights the importance of this legume to the region. Its utilization for inclusion in human as well as animal nutrition also contributes to its high productivity among small-scale farmers. The seed protein content can exceed 30% (Boukar et al., 2011) while the leaves are also rich in minerals such as phosphorus, calcium, and vitamin B (Maynard, 2008). Cowpea also has a good amino acid composition especially of phenylalanine, leucine, and lysine (Som and Hazra, 1993). Since it is a cheap source of protein, it can easily be

combined with other feed ingredients in diet formulation for ruminants (Fabian et al., 2015). However, cowpea production has declined in recent years, several factors such as pathogen and pest infection, drought, poor soil condition are some possible explanation for this decline (Boukar et al., 2013).

With a good root system, cowpea like most legumes can fix nitrogen into the soil via its root nodules. It can also serve as a cover crop against erosion and high light radiation in semi-arid regions of the world (Harrison et al., 2006). The root is an important organ in land crops, it supplies nutrients, moisture and other compounds needed for normal development of flowers, fruits and seed in angiosperm (Bonfante and Anca, 2009; el Zahar Haichar et al., 2014; Miransari, 2011). Soil acidity can limit normal growth and development of plant roots (Haling et al., 2011). The root of cowpea grows on the topsoil, however, in times of drought, they develop a taproot to aid in nutrients uptake (Jati et al., 2013).

The goal of this experiment was to observe how germination rate, leaf, root and shoot growth are affected by different pH levels and to propose an optimum pH range for cowpea's growth and development in the few weeks after emergence.

2. Materials and Methods

2.1 Collection of Sample

Seed samples were purchased from a local market in Budapest Hungary.

2.2 Experimental set up

All laboratory procedures were carried out at the Institute of Botany and Ecophysiology, Department of Agricultural and Environmental Sciences of Szent István University Gödöllő Hungary. Five different pH levels (4, 5, 6, 7 and 9) were adjusted using a benchtop pH meter. For germination experiment, perforated linen was used on top of a rack to provide firmness for root germination; this was then placed in a medium-size trough and filled with distilled H₂O (Fig 1). Germination rate was measured after 24, 48, 72 hours. The number of seeds used to determine the germination rate was 25 seeds for each pH level.



Figure 1 Germinated seeds at pH9 after 72hrs

Three germinated seeds from each of the five pH levels were randomly selected and placed in glass bottles. These 15 bottles each contained 700 ml of liquid media adjusted to the five different pH levels. The bottles containing seedlings were then transferred to a growing chamber under ambient temperature (Fig 2). Seedlings were placed at random, and the liquid media was refilled at 5 – 6 days interval.



Figure 2 Plants in the growth chamber

Following three weeks in the growing chamber, the shoot length (Ls) and root length (Lr) of fresh samples were recorded using a meter rule. The number of leaves (Nl) was also noted. Shoot, roots, and the number of leaves from each pH level were then placed in a drying bag and transferred to an oven. After 24 hours of drying at 60°C, the shoot mass (Mb), roots mass (Mr) and leaf mass (Ml) were measured in grams using a weighing balance. Dry masses were measured in grams for each pH level with replicates and the average of each Mb, Mr, Ml, Lr, Ls and Nl was recorded and used to construct charts and graphs. Lr and Ls were measured in centimetre.

The mass per meter of the root was assigned as gR, this is a ratio between root mass (Mr) and root length (Lr) and can be used as an indicator of root growth.

$$gR = \frac{Mr}{Lr} * 100$$

2.3 Media preparation

Macro and micro elements needed for optimum root and shoot growth and development were combined to facilitate nutrient uptake by the roots. Further, in another solution, the pH of media was adjusted using a benchtop pH meter. The acidic pH was achieved using hydrogen chloride (HCl) at a concentration of 33%. There was no adjustment needed for neutral pH7. On the other hand, sodium hydroxide (NaOH) was used to reach the alkaline pH (pH9). Distilled water was used for the preparation of all liquid media. Other materials used for preparing the media include

2-liter beaker, weighing balance for measuring the mass of chemical compounds, graduated cylinder, magnetic stirrer, and pipettes.

Table 1 shows the macro and micro nutrient composition used to prepare the liquid media. Five different stock solutions (**A**, **B**, **C**, **D**, and **M**) were prepared.

Table 1 Macro and micronutrient stock solution

Macronutrient stock solution		Mol wt	mM	g/100ml	Stock ml		ml/5liter
A	KNO ₃	101.11	5	10.11	100ml	A	25
B	KNO ₃ Ca(NO ₃) ₂ x 4H ₂ O	236.15	5	23.62	100ml	B	25
C	K(H ₂ PO ₄)	136.09	1	13.61	100ml	C	5
D	MgSO ₄ x 7H ₂ O	246.48	2	24.65	100ml	D	10
M Micronutrient stock solution				g/250ml	250ml	M	5
	MgCl ₂ x 4H ₂ O	197.91	11	0.5443			
	ZnSO ₄ x 7H ₂ O	287.54	4	0.2875			
	CuSO ₄ x 5H ₂ O	249.68	0.8	0.0500			
	(NH ₄) ₆ Mo ₇ O ₂₄ x 4H ₂ O	1235.86	0.5	0.1545			
	H ₃ BO ₃	61.83	47	0.7265			
	Fe-EDTA			0.6250			

Mol wt = molar weight

Table 2 shows the concentration of hydrochloric acid and its dilution to arrive at the required pH of 4, 5 and 6, also the two stock solutions here (**G**, **H**) were used to adjust the pH to acidic level.

Table 2 Chemical compound used to reach acidic pH

Stock solutions		Mol wt	mM	µl/500ml	Stock ml		ml/500ml	pH	ml/250ml
G	HCl (cc. 37%)	36.46	10	417	500ml			2	
H	HCL (10mM, pH = 2)		0.1			G	5	4	2.5
	HCL (0.1mM, pH = 4)		0.01			H	50	5	25
	HCL (0.1mM, pH = 4)		0.001			H	5	6	2.5

Table 3 shows the dilution of the sodium hydroxide to reach the optimum pH9. Stock solution **N** was used in the final media.

Table 3 Chemical compound used to reach alkaline pH

Stock solutions		Mol wt	mM	g/250ml	Stock ml	ml/500ml	pH	ml/250ml
N	NaOH	39.997	10	0.1000	250ml		12	
O	NaOH (10mM, pH = 12)				N	5	10	2.5
	NaOH (0.1mM, pH = 10)				O	50	9	25

Table 4 shows the final media concentration as well as the proportion of the different stock solutions (**A, B, C, D, G, H, M, and N**).

Table 4 Amount and concentration of liquid media

Stock	pH4 ml / liter	pH5 ml / liter	pH6 ml / liter	pH7 ml / liter	pH9 ml / liter
A	5	5	5	5	5
B	5	5	5	5	5
C	1	1	1	1	1
D	2	2	2	2	2
M	1	1	1	1	1
G	10	1			
H			10		
N					1

2.4 Statistical analysis

Statistical analysis was performed using R program version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>) and Microsoft 365 Excel package. Correlations, averages, tables, and figures were done using this software. Shapiro-Wilk test of normality and Pearson's correlation was also performed to observe the relationship between traits.

3. Results

3.1 Germination result

Table 5 shows the germination rate after three different hours. At 24 hours, pH4 recorded the highest germination percentage of 36% while pH9 had the lowest rate of just 24%. At 48 hours, pH4, pH7, and pH9 all reached 92% germination rate, while all pH level recorded a 100% germination rate after 72 hours.

Table 5 Germination rate in percentage

Hours	pH (%)				
	4	5	6	7	9
24	36	28	28	32	24
48	92	84	80	92	92
72	100	100	100	100	100

3.2 Test for normality and correlation of variables

Shapiro-Wilk test of normality was performed, and all variables were normally distributed ($P < 0.05$), hence Pearson's correlation was used to observe relationship and significance between NI, Lr, Ls, Mr, Mb, MI and gR. A strong negative correlation was found between NI and Lr (Fig 3) with high significance $P < 0.01$. Also, Lr was negatively correlated with all other traits measured.

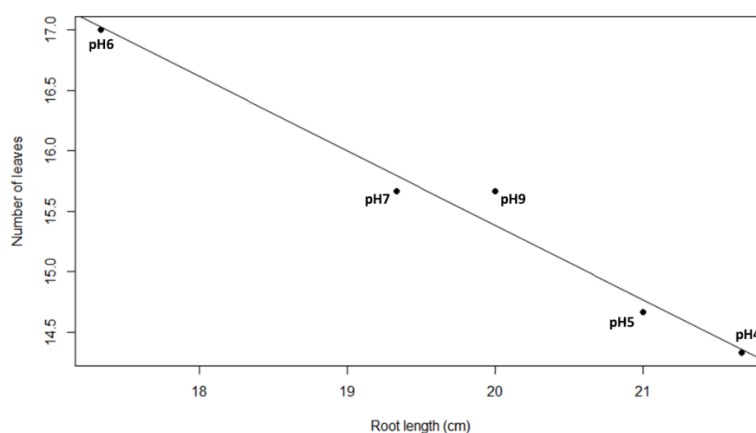


Figure 3 Correlation between root length and number of leaves

Furthermore, strong positive correlations were also reported between Mr and Mb, as well as Mr and MI with a significant difference ($P < 0.05$) (data not shown). Also, Mr was positively correlated with gR (Fig 4) and showed high significance ($P < 0.01$). Ls had no significant effect on any traits measured.

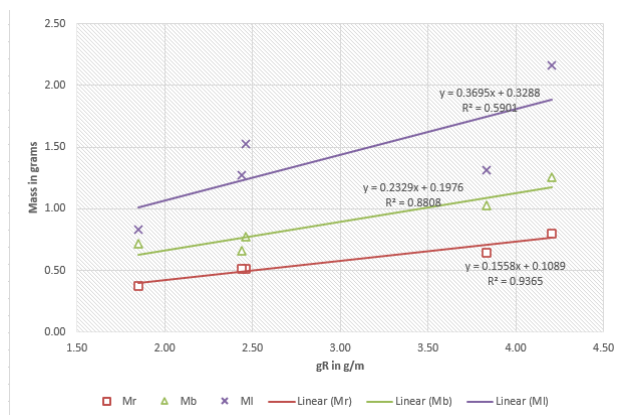


Figure 4 Relationship between gR with Mr, Mb, and MI

3.3 Distribution of variables according to different pH level

The highest masses for shoot, root and leaves were found in pH7, on the contrary, the least mass for the same traits was recorded in pH9 (Fig 5).

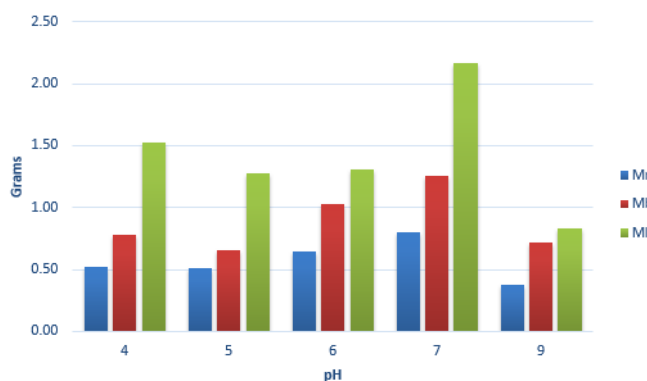


Figure 5 Effect of pH on the root, shoot and leaf mass

Fig. 6 below shows the distribution of NI and MI against different pH levels. The highest and lowest NI was observed in pH6 (17.00) and pH4 (14.30) respectively. Noteworthy, the highest MI was recorded in pH7 with a mass of 2.16g while pH9 had the lowest mass of 0.83g, yet both pHs had the same number of leaves (Fig 3). There was no significant difference between the leaf mass of pH5 and pH6.

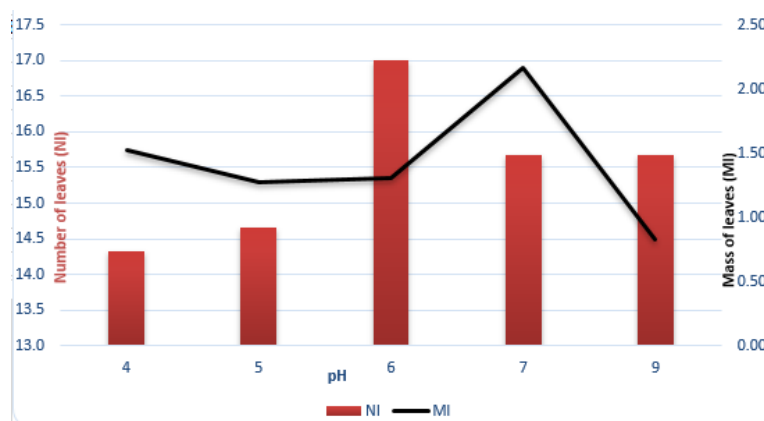


Figure 6 Effect of pH on number of leaves and mass of leaves

4. Discussion

The range of the germination rate after 24 hours is between 24 – 36%, with slightly acidic pH4 having the highest rate while the alkaline pH9 had the lowest. In a similar study carried out by Gentili et al. (2018), also reported higher germination rate among slightly acidic pH levels compared to the alkaline pH. From this study, there was no significant difference of pH on the germination rate after 72 hours.

The negative correlation between NI and Lr (Fig 3) shows that high vegetative cover (number of leaves) can negatively affect root growth in early plant development. It has been recommended that the early weeks of root growth should be promoted for optimal growth and development of plants (Duncan, 2000). However, this correlation could be beneficial when designing pasture for ruminant or other livestock species since they require high protein content obtained from leaves and shoots whereas root growth is not of great importance in the pasture for ruminants. This finding can be an added knowledge to low-income farmers in developing countries who are into pastoral farming as they require less nutrient to achieve optimal crop yield.

Despite pH7 and pH9 having the same number of leaves, yet the masses of their leaves were greatly different, pH7 was 2.5x that of pH9 (Fig 6). A few weeks after emergence, the leaves of plants are mostly made up of water (Goel and Rao, 2004). This implies the role of moisture contents in the different pH levels. Therefore, for diet formulation and pasture design, leaf mass and not leaf cover is a reliable indicator for legumes grown for pasture. Kamaluddin and Zwiazek (2004) reported that low pH results in a rapid decline of water flow as well as hydraulic conductance in roots of seedlings of paper birch. This could explain why although pH7 and pH9 had the same number of leaves, but their dry mass is widely different.

Furthermore, the high correlation between Mb and gR means that gR explains 88% of the variation in Mb. In other words, improving root growth will subsequently result in improved shoot growth.

5. Conclusions

This experiment has confirmed that there is no significant difference of pH on germination rate in cowpea specie, however, pH plays a role in aerial emergence and showed greater variation in the root, shoot, and leaf mass, as well as shoot and root length including the number of leaves. From this experiment, associations between different traits were established. This will be important for selection in breeding or explaining the association of traits. For optimal growth of roots, shoots and leave during the early weeks of cowpea emergence, pH7 is therefore recommended. To consolidate on the findings from this study, future research should include more seed samples and growth should be monitored up to flowering time to observe other interesting relationship which might be of promising to local farmers and pastoral farmers.

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