Establishing Nutrient Efficient Hydroponic Protocol for Cowpea (Vigna unguiculata L. Walp) Evaluations at Seedling Stage

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Abstract: Hydroponic growth systems are a convenient platform for studying whole plant physiology. Major yield loss in cowpea (Vigna unguiculata) can be attributed to biotic and abiotic stresses. A cost effective and time saving screening protocol for cowpea abiotic stresses will enhance and expedite research on cowpea by breeders thereby enhancing its production and availability to meet increasing demand and population rise. Two nutrient formulations (NFZ1 and NFZ2) and a control were evaluated upon considerations of cowpea (16 varieties) growth requirement on soil. These treatments consisted of variations of the combinations of N-P-K fertilizers, FeSO4 and ZnSO4. Seedling biometrics after 30 days in hydroponics indicated that NFZ2 induced greater seedling performance than NFZ1. This increase in growth rate ranged from 12.83% to 65.24% adopting the NFZ2 protocol in slow and fast growing varieties, respectively with an average growth rate of 46.35%. Therefore, this protocol is recommended where fast screening for cowpea is desired in evaluations for breeding purposes.

Key words: Cowpea, Vigna unguiculata, Evaluation protocol, Hydroponics.

INTRODUCTION

Cowpea (Vigna unguiculata L. Walp) is a warm seasonal annual herbaceous legume belonging to the family Fabaceae and sub family Papilionaceae (Davis *et al*., 1991). It serves as a cheap source of protein for most people in many developing countries where per capital income and intake of animal protein are both very low (Okigbo, 1976). Cowpea contains 200 – 300g crude protein and 600g of carbohydrate per kilogram (kg) seed. The chemical composition is influenced by environmental and genetic factors (Singh, *et al*., 2006). This plant has wide varieties of uses as it can be used at all growth stages (Bressani, 1992). Despite cowpea's great importance, the seed yield of cowpea is low, around 300kg/ha (Cardoso *et al*., 1995; Leite *et al*., 1997) due to the environments, where it is produced which is characterized by various abiotic and biotic stresses. A cost and time effective screening protocol for cowpea will enhance and expedite research on cowpea by breeders thereby enhancing the production of good quality cowpea which in turn contributes to its availability in meeting demand. In this context, hydroponics is a major scientific modelling tool, facilitating precise control over the treatment and consistent observations of treatment effects. In order to perform such assays whilst providing a flexible experimental platform for manipulation of both the shoot and root environment, the use of hydroponics for research purposes has become common (Gregorio *et al*., 1997). Importantly, hydroponics enables observations to be made of intra- and interspecific genetic variation in plant responses. Hydroponics, the 'water culture' of plants, has been used in both research and commercial contexts since the 18^{th} century. Although now used successfully on a large scale by commercial growers of fast-growing horticultural crops such as lettuce, strawberries, tomatoes, and carnations . Hydroponics was initially developed as a part of early research into plant nutrition. The use of hydroponics enabled plant scientists to identify which elements were essential to plants, in what ionic forms, and what the optimal concentrations of these elements were. It allowed them to easily observe the effects of elemental deficiencies and toxicities and to study other aspects of plant development under more controlled (temperature- and light-controlled glasshouses) conditions (Yuri *et al*., 2012).

Hydroponics has been instrumental in establishing the essentiality of most of the mineral nutrients required by plants (Jones, 1982; Reed, 1942), from the early development of nutrient solution recipes in the 1860's by the German scientists Sachs and Knop (Hershey, 1994), through to when nickel was confirmed as an essential micronutrient for higher plants (Brown *et al*., 1987). It is particularly useful in identifying visual symptoms or critical deficiency concentrations for diagnostic purposes, characterizing physiological functions of mineral nutrients, determining their uptake kinetics, studying root exudates and gene expression changes and also changes in root morphological traits in response to nutrient deficiencies. It is also commonly used to identify germplasm with enhanced nutrient use efficiency (that is, an ability to produce greater biomass at limited nutrient supply) for breeding programs.

However, the use of hydroponics is limited due to trailing contaminations It is particularly useful in identifying visual symptoms or critical deficiency concentrations for diagnostic purposes, characterizing physiological functions of mineral nutrients, determining their uptake kinetics, studying root exudates and gene expression changes and also changes in root morphological traits in response to nutrient deficiencies. It is also commonly used to identify germplasm with enhanced nutrient use efficiency (an ability to produce greater biomass at limited nutrient supply) for breeding programs (yuri *et al* 2012; Conn *et al*., 2013).

MATERIALS AND METHODS

Study Area and Cowpea source

The present study was carried out at the screen house of the Department of Biological Sciences, Ahmadu Bello University (Long. $07^o – 38^oE$ and Lat. $11^o – 11^oN$) Zaria, Kaduna State, Nigeria. Sixteen varieties of cowpea were obtained from the Institute of Agricultural Research (IAR) Zaria, Kaduna State, Nigeria.

Hydroponic system set-up

The hydroponic set up was based on Gregorio *et al*., (1997) recommendations for rice hydroponic protocol with modifications. The equipment (plastic tanks, styrofoam sheets, wire mesh) were dark coloured (except for the styrofoam sheets) to minimize light penetration into the culture solution, thus reducing algal growth. The plastic tanks had an outside dimension of 35 x 29 x 14 cm and contain approximately 8.6 litres of water (Plate 1). The size of tank can be changed to suit local conditions. The support platforms used was a wire mesh of mesh size 0.3cm, glued to fit the bottom of the styrofoam sheet with a glue gun (plate 2). The Styrofoam sheet (contain 15 circular compartments (4 x 4cm) cut at regular intervals with a spacing of 3cm) can accommodate 15 seed (one seedling per hole) and overlaps the plastic tank.

The wire mesh attached to the bottom of the styrofoam is to hold the seed and seedling and prevent them from sinking in the growth media. This set-up allows for constant aeration of the hydroponics system, thus, preventing the system from being anaerobic.

Composition of nutrient solutions

All nutrient solutions used for hydroponics culture of plants are essentially formulated based on plant growth requirement. The growth solution consisted of the following essential macro-elements: nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg) and sulphur (S); and micro-elements: a soluble form of iron (Fe), zinc (Zn) and chlorine (Cl). Final concentration of elements per litre of distilled water were as follows: (i) 1.5g/L of N-P-K (15-15-15) fertilizer + 0.2g/L of $FeSO_4$ +0.05g/L $MgCL_2$ +0.2g/L $ZnSO_4$ - (NFZ_1), (ii) 1.5g/L of N-P-K (20-10-10) fertilizer + 0.2g/L of $FeSO_4$ + 0.05g/L $MgCL_2$ +0.2g/L $ZnSO_4$ - (NFZ_2) and (iii) 0.2g/L of $FeSO_4$ + 1.5g/L Peters 20-20-20 water soluble fertilizer (Control).

Germinating seedlings

The styrofoam sheets were placed on a four corner plastic tank. Tanks were filled with distilled water until the water level is about 1mm above the mesh. Two seed of cowpea was directly sown per hole on the wet Compartments on the Styrofoam sheet. Seeds were surface sterilized prior to germination by soaking in 20% chlorox solution for 20-30 minutes, followed by three rinses in distilled water. Chlorox treatment also helps to promote germination.

The experimental set-up was covered with a perforated (to allow for continuous aeration) black plastic bag to facilitate positive root geotropism. After 96 hrs, when the growth of the root towards the growth media was observed, the plastic covering was uplifted to allow for photosynthesis of the emerging cotyledons. At this stage the plant was thinned down to a single plant per hole to avoid overcrowding. Four days after seeding, the various treatments were added and stirred into the tanks containing distilled water. The pH was maintained daily at 5.0 ± 0.2 using 1N HCl and 1N NaOH. Due to evaporation and transpiration, pH change (algal growth may also contribute in pH fluctuation) and loss of solution volume were made up by

ISSN 2229-5518
adjusting the PH and by addition of distilled water. This was implemented daily. The pH was adjusted by lifting off the Styrofoam sheets and placing them temporarily onto empty tanks before adding HCl/NOAH to regulate the pH. Once adjusted, the nutrient media was stirred before replacing back the Styrofoam sheet. The screening was done in glasshouse conditions with day/night temperatures of 30/20ºC and relative humidity of at least 50% during the day. The glasshouse was disease free and well lit by natural lighting. The tanks were placed on a levelled floor.

The research work consists of two treatments, set up in a complete randomized design, with each treatment replicated thrice and a control medium for each treatment.

Data were collected on the (i) Seedling height (cm), (ii) Number of leaves per seedling: (iii) Leaf diameter and (iv) Leaf chlorophyll content (μg. $mgdw^{-1}$). Data was subjected to analysis of variance (ANOVA) with mean separation ($P > 0.05$) by Duncan's Multiple Range test (DMRT).

RESULTS

Hydroponic System

The hydroponic system supported the vegetative growth of cowpea. The system enhanced accessibility to all plant tissues and enabled easy manipulation of the nutrient profile of the growth medium. The Styrofoam holes, mesh, dark colour of the mesh and growth tank, and the continuous daily aeration by stirring of the growth media aerated the nutrient solution, hence algal contamination (10% in NFZ1, 5% in NFZ2 and 20% in control) and hypoxia was minimal.

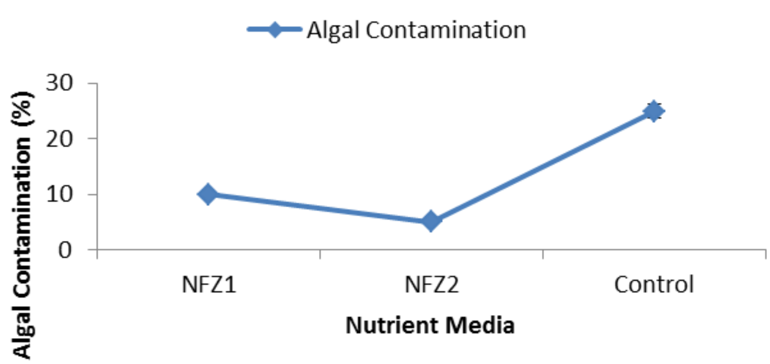

Figure1: Percentage Algal Contamination in Hydroponics system due to Nutrient Media.

Plant growth

A number of measurements were made to ascertain the growth response of plants grown in our hydroponic system. The growth parameters of cowpea seedlings as influenced by difference in the growth media are presented in Table 1. Under the hydroponic conditions using the NFZ1, NFZ2 and Peter's water soluble fertilizer, plants had vibrant green colouration. The growth rates throughout the vegetative growth significantly (p> 0.05) differed. NFZ2 nutrient formulation positively and significantly affected the plant height of cowpea seedlings relative to NFZ1 and control. This increase in growth rate (shoot height) ranged from 12.83% to 65.24% adopting the NFZ2 formulation in slow and fast growing varieties respectively with an average increase in growth rate of 34.37%. NFZ2 produced plants with the highest plant height and chlorophyll content, this was closely followed in descending order by NFZ1 and the control. The numbers of leaves per plant and leaf diameter were not significantly affected by the nutrients. The total mean chlorophyll content of 4-week old hydroponics plant leaves was 13± 0.4 µg.mgdw^{-1}, 15.5 ± 0.6 µg. mgdw^{-1} and 12.5 ± 0.4 µg. mgdw^{-1} for NFZ1, NFZ2 and Control respectively (figure 2).

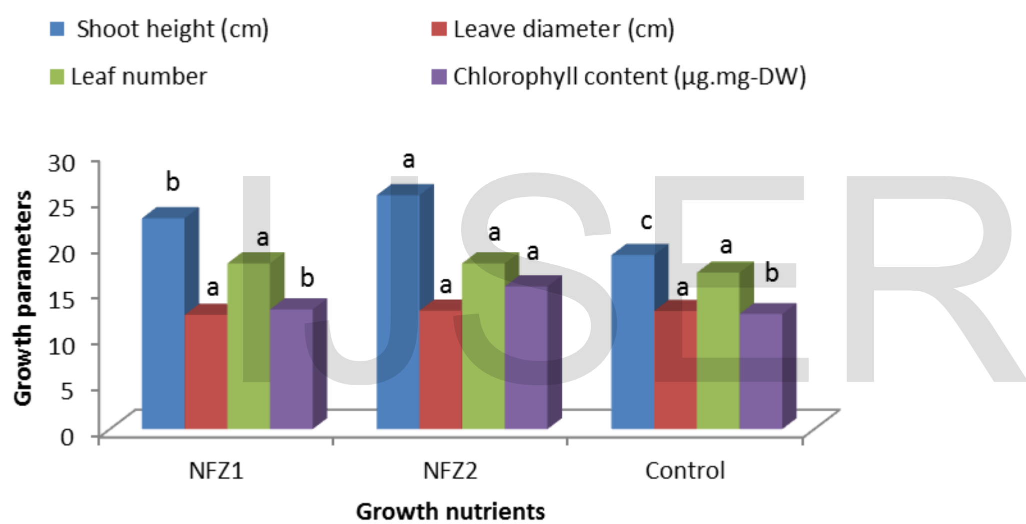

Figure 2: Effect of treatments on the mean growth (shoot height (cm) and Leave diameter (cm) of sixteen cowpea varieties.

Table 1: Effect of treatments on the shoot height (cm) and Leave diameter (cm) of sixteen cowpea varieties.

Cowpea Varieties	Shoot Height (cm)			Leave Diameter (cm)		
	NFZ1	NFZ2	CONTROL	NFZ1	NFZ2	CONTROL
Yar Waja	22.75±1.06^{d}	27.20±1.27^{b}	20.30±0.56^{b}	12.50±2.12^{b}	12.50±2.12^{b}	12.50±2.12^{b}
Kanannado Brown	24.50±2.12^{c}	24.50±0.57^{cd}	19.50±0.42^{bc}	14.00±0.11^{a}	12.50±2.12^{b}	12.50±1.87^{b}
Daroje	24.55±0.78^{c}	26.30±1.57^{bc}	19.55±0.78^{bc}	11.00±0.21^{c}	11.00±0.18^{c}	14.00±1.44^{a}
Iar-339-1	23.25±0.35^{cd}	25.05±0.78^{c}	22.50±4.67^{a}	11.00±0.22^{c}	14.00±1.11^{a}	11.00±0.58^{c}
Danwuri	24.40±0.14^{c}	25.20±0.85^{c}	22.50±0.56^{a}	14.00±0.56^{a}	14.00±1.08^{a}	12.50±1.18^{b}
Bodeje	20.65±1.63^{e}	24.70±2.40^{cd}	15.80±0.85^{de}	12.50±2.12^{b}	12.50±2.18^{b}	12.50±2.01^{b}

ISSN 2229-5518

IAR-353	21.15±0.35^{de}	22.05±1.48^{d}	19.10±0.91^{bc}	12.50±2.12^{b}	12.50±2.12^{b}	14.00±0.55^{a}
IT90K-277-2	20.05±0.78^{e}	21.90±1.70^{de}	18.20±0.57^{c}	11.00±0.24^{c}	12.80±2.12^{b}	12.50±1.14^{b}
Sa babba sata	19.15±0.64^{f}	17.15±2.48^{e}	15.20±0.28^{de}	14.00±1.10^{a}	14.00±0.58^{a}	14.00±4.23$_{a}$
IT 89KD-288	22.70±0.57^{d}	25.85±0.64^{c}	18.80±0.71^{c}	14.00±0.98^{a}	12.00±1.87^{b}	11.00±1.11^{c}
IT 93K-452-1	27.15±1.20^{b}	27.75±0.78^{b}	20.10±1.41^{b}	14.00±0.12^{a}	11.00±0.48^{c}	14.00±1.55^{a}
IAR-355	19.45±1.20^{f}	25.15±0.92c	13.95±0.64^{e}	11.00±0.41^{c}	12.50±2.12^{b}	12.50±0.98^{b}
Nabbewela	26.70±1.84^{bc}	31.30±2.55^{a}	21.15±1.63^{ab}	11.00±1.11^{c}	14.00±0.47^{a}	11.00±1.18^{c}
Bahaushe	21.15±1.63^{de}	24.55±1.06^{cd}	19.20±0.42^{bc}	12.50±2.12^{b}	14.00±0.41^{a}	14.00±1.62^{a}
IAR-48	29.05±1.34^{a}	32.80±1.27^{a}	19.85±1.34^{bc}	11.00±0.56^{c}	12.58±1.88^{b}	12.50±2.00^{b}
MaiBargo	19.95±0.71^{f}	25.10±0.42^{c}	16.85±1.34^{d}	12.50±1.18^{b}	14.00±1.11^{a}	14.00±1.27^{a}

Treatment means within each column followed by the same letter are not significantly different from each other using Duncan multiple range test at 5% level.

Discussions

Hydroponic, as a convenient means for studying plants in the laboratory and for growing commercial crops, was a term first coined by William F. Gericke in 1929, yet it is a documented technique dating back to the late 17th century (Hershey, 1994). Many hydroponic systems for various plants have been reported (Berezin *et al*., 2012). Its advantages include the potential for accessibility to all plant tissues and the easy manipulation of the nutrient profile of the growth medium when compared to soil, given the complex interaction of ions with soil particles. The idea of hydroponics, its development and improvement, stimulated our interest to provide an efficient system for cowpea growth and development for unfavourable (toxicity/deficiency) growth environments. We presented a simple, inexpensive, flexible and robust hydroponics system for the cultivation of cowpea. The flexibility of our hydroponic system makes it amenable for applying many morphological, physiological and molecular analyses of the plant tissues.

Plant growth and development are dynamic processes that can be perturbed by a number of biotic and abiotic factors, including nutrient availability, oxygenation of growth solutions, prevalence of microorganisms, humidity and air temperature (Mündermann *et al*., 2005). Coosemans, (1995) reported that one common and significant problem associated with aggregate hydroponics growth systems is the algal contamination of the culture medium. This can occur in the tank, and particularly on rockwool or agar-based plugs, or the plant roots and shoots, due to the use of non-sterile phosphorous-rich medium and the exposure of these components to light. The dark growth tank and mesh used reduced illumination to the growth media thus, reducing algal growth. Furthermore, aeration of the growth media eliminated hypoxia and improved plant growth hydroponically. Therefore, proper aeration of growth media, and the use of dark growth equipment's to eliminate light illumination is essential for hydroponic growth of cowpea.

Our nutrient media were simple and affordable. They were based majorly on inorganic fertilizers. Fertilizers are organic or inorganic material of natural or synthetic origin (other than liming materials) that is added to a soil to supply one or more plant nutrients essential to the growth of plants (Stewart, 2005). Nutrient media is an environment designed and enriched with nutrient elements to support the growth of plants. Plant nutrients are mostly inorganic or ionic forms of chemical element. There are two types of nutrients, viz: micronutrients, which are nutrient elements required by plants in trace amount for example, Iron(Fe), Manganese (Mn), Copper (Cu), Molydenum (Mo), Boron (B); and macronutrients (Nitrogen (N), Phosphorus (P), Potassium (K), Sodium (Na), Calcium (Ca)), which are needed by the plants in large amount (Lyons et al., 2009) . Of these, the three most important ones are the Nitrogen (N), Phosphorus (P), and Potassium (K). Although some macro and micro nutrients were lacking in our nutrient formulations, they were able to support plant growth. This suggests that cowpea can grow optimally in nutrient deficient environments. Scientists discovered that plants required only a small number of inorganic elements, in addition to water, oxygen and sunlight, to grow (Yuri *et al*., 2012). The preferential response of the cowpea varieties to the nutrient formulations, focusing on NFZ1 and NFZ2 could be attributed to the percentage concentrations of the individual elements (nitrogen, phosphorus and potassium). The high growth rate (shoot length) and chlorophyll content of the NFZ2 exposed cowpea varieties was indicative of its higher nitrogen content. Similarly, (Genc *et al*., 2007) observed that the growth of bread wheat seedlings was significantly reduced (37%) at 5 mM NH_4+ compared to 1 mM NH_4+ when grown in nutrient solution containing 5 mM KNO3 / 2 mM $Ca(NO3)_2$.

Conclusions: We present a nutrient efficient and optimized plant hydroponic system that can be quickly and cheaply constructed, and support the growth of cowpea with the advantage of being a versatile platform for a myriad of physiological measurements on all plant tissues at all developmental stages. There-in are growth requirements and conditions of the hydroponic system.

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