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Making water stress treatments in pot experiments: An illustrated step-by-step guide

Heliyon

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Abstract

This protocol is a step-by-step guide on how to make water stress treatments in pot experiments. Water stress treatments are useful for investigating how plants survive under drought conditions. This protocol demonstrates how you can keep plants severely water stressed, moderately water stressed and well-watered. Note that water stress is sometimes also referred to as soil moisture stress or simply as moisture stress. The terms will be used interchangeably in this protocol. Water stress treatments are made by reducing a soils moisture content and by maintaining the moisture content at this new low level for a period of time. Changes in soil moisture are achieved by reducing or increasing a soils moisture content to levels close to or way below its field capacity (water holding capacity). Field capacity is the maximum amount of water that a soil retains after the excess water it had gained from an irrigation or rainfall has completely drained and its percolation seized. Note that moisture content at field capacity is not the same as that held under saturated soil moisture conditions. At field capacity the large soil pores are filled with both air and water, while the smaller pores are still full of water. While at saturation all pores are filled with water. Plants need both air and water in soil pores for them to grow well making field capacity an ideal soil moisture level. There are many methods for creating water stress treatments. The method described in this protocol is however suitable for all situations.

Materials

- Air-dried soil
- Oven
- Small aluminium cans
- Three disposable 1.5 - 2 L bottles (with at least a 20 cm height)
- Knife
- Scissors
- Plastic sheets
- 5 L plastic pots; needed to hold 5 kg of potting soil
- Saucers
- Rubber bands
- Measuring cylinders (500 ml and 250 ml with accuracy of ± 2 ml at 20^{o}C and 10 ml with accuracy of ± 1 ml at 20^{o}C

Troubleshooting

Before start

In addition to this protocol you will need general knowledge on setting-up pot experiments. The paper by Poorter et al. (2012) provides important considerations to be taken when setting-up pot experiments. The information includes things like the selection of appropriate pot sizes or soil amounts that will not limit plant growth and interfere with treatment observations. Pot sizes must be able to accommodate the size of plants at their various development stages under different treatments. However, if particular plants are known to grow very large the length (time period) of the pot experiment may have to be shortened or larger pots should be used. Note that the increased demand for watering is an indication that pots are too small and that the amount of soil in pots cannot effectively supply the growing plant with the needed nutrients and water despite being brought to field capacity at least once in day.

Make sure that you read the entire protocol before begin preparing for your experiment. This is because, although some things only appear in a later step you will need to have prepared for them in advance. The flow of steps only makes it easier for you to understand the entire process.

1 **Determining the moisture content of the potting soil**

Air dry soil even when thoroughly air-dried always has moisture no matter how little. This moisture has to be accounted for when determining the amount of water needed to bring a soil to its field capacity. The first step thus involves the determination of the potting soils moisture content i.e. its gravimetric water content. To do this you will first have to weigh three empty aluminium moisture cans. After weighing the cans place 100 g of air-dry potting soil onto each moisture can and place the cans with soil to dry in an oven at 105°C for 24 hours. The soil should be left to dry until the cans achieve a constant weight, at this point the oven should be switched off and the cans left to cool there. When they are cool enough to be handled the cans should be removed from the oven and left to further cool in a desiccator. Once cool the moisture cans with the oven dried soil can be weighed and the moisture content determined using the equation below. The average moisture content of the three replicates needs to be calculated.

$$\text{moisture content} = \frac{(\text{mass of can} + \text{air dry soil}) - (\text{mass of can} + \text{oven dry soil})}{(\text{mass of can} + \text{oven dry soil}) - \text{mass of can}}$$

$$=> \quad \text{moisture content (g/g)} = {}^{g}/_{g}$$

$$=> \quad \text{moisture content (\%)} = {}^{g}/_{g} \times 100$$

$$\text{Average moisture content (\%)} = \frac{(\text{moisture content can 1} + \text{moisture content can 2} + \text{moisture content can 3})}{3}$$

2 **Determining the amount of air dry soil to place in each pot**

Each pot will have to be filled with an amount of soil that will give the required amount of oven dry soil. This is essential if we are to bring the soil to its field capacity or any other soil moisture level. Let us assume that we need 5 kg of oven dry soil and that our air-dry soil has a moisture content of 0.15% (0.0015g/g). What then would be the amount of air-dry soil needed to give a mass of 5 kg (5000 g) of oven dry soil? The answer is found by using the formula for determining the soils gravimetric moisture content as shown below.

Note that though the formula looks different from the one given in Step 1, it is basically the same.

$$\text{moisture content}\ (g/g) = \frac{\text{air dry soil}\ (g) - \text{oven dry soil (g)}}{\text{oven dry soil (g)}}$$

$$\text{air dry soil} = (\text{moisture content} \times \text{oven dry soil}) + \text{oven dry soil}$$

$$= (0.0015\ g/g \times 5000\ g) + 5000\ g$$

$$= 5007.5\ g$$

$$= 5.0075\ kg$$

$$\approx 5\ kg$$

From our example we can see that there is a slight difference between the amount of air dry soil needed to give 5 kg of oven dry soil. Due to the small difference only 5 kg of air dry soil will be added to each pot. This difference is however still critical and will still be accounted for when determining the amount of water to be added to the soil to bring it to field capacity. You will see this in the steps to come. **Note that**, you can carry out the same calculations using other soil weights, just replace your values with the respective values used in the examples.

3 **Determining the potting soils moisture content at field capacity**

Several methods exist for determining a soils moisture content at field capacity. The methods however have minor differences. Below I shall explain two methods. The methods were merged from two different methods that had been used to determine soil field capacity. Each individual method had aspects that were essential in the development of moisture stress treatments (check bibliography). The methods are as follows;

Method A

Steps involved:

1. Get three empty 1.5 - 2 L transparent containers. Used water bottles can be used, they must however be cut at the point where they begin to narrow as the container must have a consistent diameter throughout. Also make sure that the bottles are each at least 20 cm high after being cut. In fact, it is best for whatever container you shall use to be at least a 20 cm high. Three containers are needed, simply for replication of results.

2. Make small holes in the bottom of the containers. These will be outlets for displaced soil air by added water and they will also be outlets for percolating water. Use a knife to make small slits/holes in the bottom of the container. The holes should not allow soil to pass and they must also not hold back percolating water. The slits should be big enough to allow water and air to freely pass through them.

3. Fill the containers with the air dry soil that will be used in the pot experiment, but leave at least the first 5 cm close to a containers opening free of soil.

4. Tamp the soil in the container. You can hold the container with both hands and hit its bottom on a floor or table at least twice when tamping it. Tamping helps pack soil particles into a consistency similar to what it will have in the pot experiment.

5. Pour water over the soil surface quickly and evenly. Do not create gullies on the soil surface, while doing this. Pouring the water over the soil quickly and evenly, allows the water to create a pool over the soil surface. The pool formed allows water to infiltrate the soil evenly. The 5 cm space left free of soil helps to hold the pool of water. You can also place a filter paper or small cloth over the soil surface to ensure that the water infiltrates evenly into the soil. Keep pouring the water over the soil until the container begins to leak from the bottom (Fig 1). The leaking indicates that all the soil in the container has now been fully wetted and completely saturated. However, still check the container to see if all the soil in it looks wet, if not then continue to pour water into the container until it is all wet.

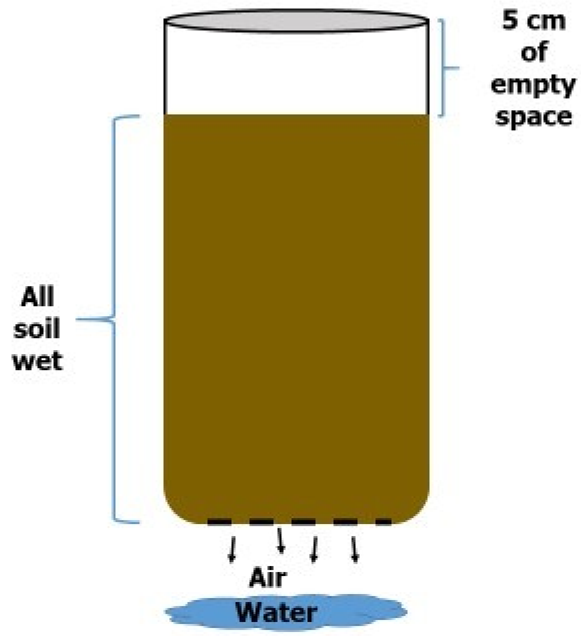

Figure 1. Container with all the soil in it made completely wet

6. Immediately cover the containers with plastic, after getting the soils saturated. The containers are covered with plastic to prevent evaporation as drainage takes place. In this way water is only lost through downward movement. Use rubber bands to securely hold the plastic sheets over the container. Place the covered containers with wet soil, away from sunlight and heat and leave them to completely drain for 3 days (Fig 2). Depending on whether a soil is coarse or fine textured it could take about 2 to 3 days for it to drain.

Figure 2. Wet soil in containers left to drain for 3 days

7. After three days collect a 100 g sample of soil from each container for moisture determination. The moist soil should be collected from the middle of the container from a distance of about 5 - 10 cm below the soil surface. Immediately place the moist soil on an already weighed moisture can and follow the steps for determining gravimetric moisture content (NB: as explained in Step 1).

Method B

Steps involved:

1. Follow **Steps 1 to 4** as written in Method A.

5. In this method only two-thirds of the soil in the container is made wet (Fig 3). This method tries to imitate soil saturation under actual field conditions, where the entire soil profile is not made completely wet despite the soil in the topsoil having been saturated by an irrigation or rainfall event. About 100 ml of water is added to the soil in each container. Slow infiltration with a pool of water created above the soil surface is still necessary. A clear wetting front develops between wet and dry soil as the water moves through the container. The wetting front stops moving through the soil when the water stops draining. Two-thirds of the soil should be wetted by the time the wetting front stops moving. As water displaces air from the soil, only air and not water escapes through the bottom of the container in this method.

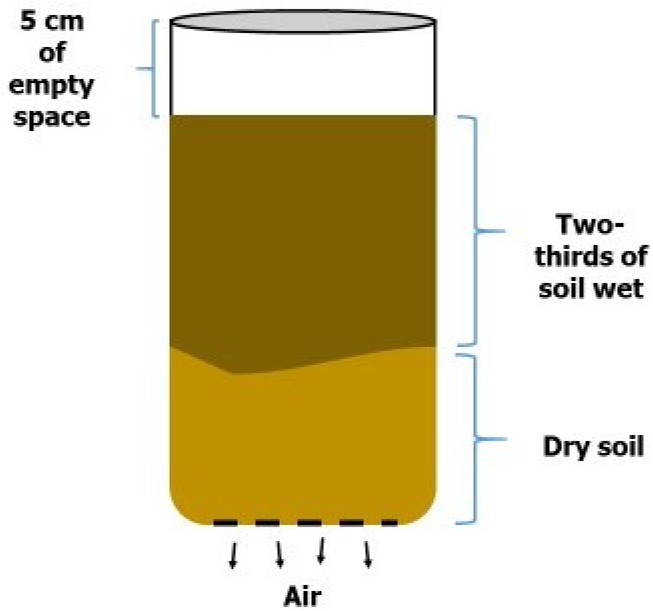

Figure 3. Container with only two-thirds of the soil made wet

6. Follow **Steps 6 to 7** just as indicated in Method A. The moist soil in this method should however be collected from about 5 cm above the wetting front.

4 **Determining the amount of water needed to bring a mass of potting soil to field capacity**

In the previous Step we saw how to determine a soils field capacity. Let us assume that Method A gave us a field capacity (moisture content) of 18.7% while Method B gave 12.6% (these are actual results and thus reflect how the two methods perform). These values constitute the full amounts of water needed to bring the soil to 100% field capacity. Note that one method gave a higher required field capacity than the other. This should not worry you, you shall later appreciate the purpose of comparing the two methods. Only one method is however needed. Remember that you will be adding a certain mass of air dry soil to each pot and that the air dry soil is an equivalent mass of oven dry soil. Also remember that the air dry soil contains moisture. This amount of moisture needs to be accounted for no matter how small it might seem to be. We shall now demonstrate how it should be accounted for using the result for **Method A** as follows:

The amount of moisture needed to bring the soil to field capacity is 18.7% (0.1867 g/g). This means that each gram of soil will need 0.1867 g of moisture to bring it to field capacity. However, remember that we shall be working with an air dry soil sample which already contains 0.15% moisture. The amount of water needed to bring this soil to field capacity will thus be slightly less than 18.7% as shown below.

$$\text{Amount of water needed to bring soil to field capacity}(\%)$$
$$= \text{moisture content at field capacity}(\%) - \text{moisture content in air dry soil}(\%)$$
$$= 18.67\% - 0.15\%$$
$$= 18.52\% \ or$$
$$= 0.1852 \ g/g$$

The result of the calculation thus shows that only 0.1852 g of water will be needed to bring 1 g of oven dry soil to 100% field capacity, considering that 0.0015 will be provided by the air dry soil. We now have to calculate the amount of water needed to bring our 5000g (5 kg) of oven dry soil to 100% field capacity, without the water provided by the air dry soil. This is shown below as follows:

$$\frac{1\ \text{g oven dry}\ soil}{5000\ \text{g oven dry}\ soil} = \frac{0.1852\ \text{g of}\ water}{X}$$

$$=> \quad X = \frac{0.1852\ \text{g of water} \times 5000\ \text{g oven dry}\ soil}{1\ \text{g oven dry}\ soil}$$

$$=> \ X = \frac{0.1852\ \text{g} \times 5000\ \text{g}}{1\ \text{g}}$$

$$= 926\ \text{g of water}$$

While expressing the needed amount of water as a mass is useful for calculating how heavy the final mass of soil plus water will be, it not useful for telling us how much water must be added as a volume. Water is much easier to add in volumes than as a weight. Now you may recall that the density of water is 1 g/cm^3. The equivalent volume of water can thus be calculated as shown below:

$$\text{volume} = {}^{mass}/_{density}$$

$$= \frac{926\ \text{g of}\ water}{1\ \text{g}/cm^3}$$

$$= 926\ cm^3\ \text{or}\ 926\ \text{ml}$$

When you carry out the same calculations using the field capacity obtained for **Method B** you will find that 622.5 ml of water is needed to bring 5000 g of soil to field capacity when you take into account the moisture that will also be added from the air dry soil. You can test the two methods to see which is more suitable. You can do this by taking two identical 5 L plastic pots filled with 5007.5 g (≈ 5000 g) of air dry soil. Cover the holes in the bottom of the pot with cotton wool before placing the soil in them. Note that pots will also be prepared in this manner during the actual experiment. Cotton wool is light and porous and it will block the holes while still allowing air and water to pass through. Do not forget to place a saucer below the pot. Pour each respective amount of water into a pot i.e. 926 ml in one pot for **Method A** and 622.5 ml in the other pot for **Method B**. Cover each pot with plastic to prevent evaporation and leave them to completely saturate and drain for 24 hours.

After 24 hours, examine the pots to see if any water has leaked out onto the saucer. Leaking would indicate that the calculated amount of water is above field capacity and thus too much. However, you will only be able to know if the water is too little by examining the wetted soil in the test pots. The soil from the pots will need to be taken out from each pot and placed onto a saucer. The soil should be removed as a complete column of soil in an upside-down position (Fig 4). To do this you should first place the saucer above the opening of the pot and while holding it firmly, quickly turn the pot upside down and carefully lift-off the pot so that you leave behind only the column of soil resting on the saucer. Examine the column of soil from each pot for any dryness. If one pot has dry soil on its upper end, it means that the water did not fully saturate the soil and was thus insufficient to bring the soil to field capacity (Fig 4). Note that part of the soil column may stick to the bottom of the pot if the soil is too wet. A soil that is well saturated will be fully wet, with no leaking and will leave the walls of the pot clean but moist as if they had been wiped with a damp cloth. Of the two methods I found **Method A**more suitable. **Method B** gave less than the required field capacity moisture but this could have been because the drainage time (3 days) that I used when determining field capacity (Step 3) was longer than that recommended in the original method (i.e. 24 hours).

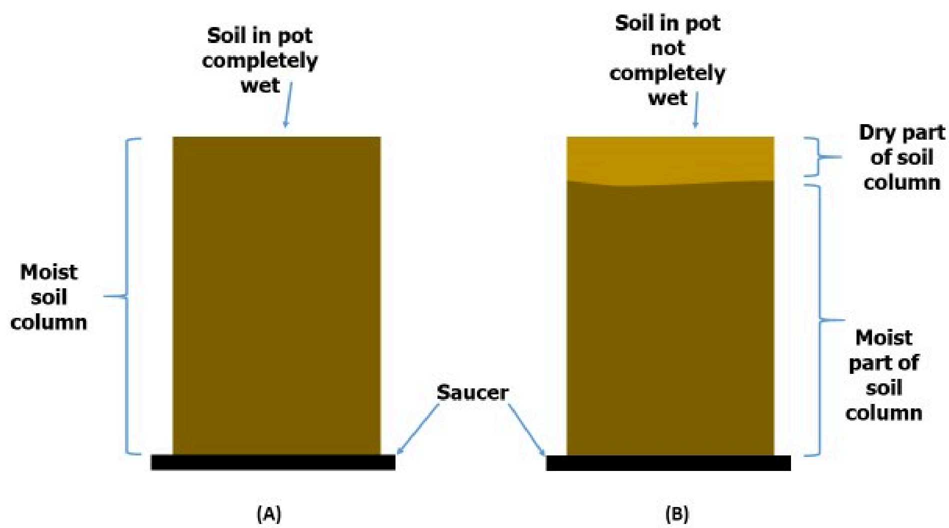

Figure 4. Columns of soil from pots, in their upside-down positions, used to test whether the calculated amount of field capacity moisture was not/enough

5 **Making plants in pots severely and moderately water stressed**

Now that we know the volume of water needed to add to our air dry mass of potting soil of 5007.5 g, to bring it to field capacity, we can now easily calculate how much water to add to the pots to achieve moderate and severe water stress. This is achieved by simply changing the percentage volume of field capacity moisture of soils in pots that will hold severely and moderately stressed plants. Severe moisture stress is often achieved by maintaining soil in pots between 20% and 30% field capacity. A field capacity of 20% is alright for fine textured soils, like the clayey soils, but is too low for coarse textured soils, like sandy soils and could kill plants growing on coarse textured soils. You off course do not want to kill the plants in your experiment, unless that is the objective of your work. Moderate water stress is often achieved by maintaining moisture in pots at 60% field capacity in both coarse and fine textured soils and 100% field capacity will keep plants in pots under well-watered conditions. Table 1 gives a summary of required percentages of field moisture at severe and moderate water stress.

Table 1: Percentage of field capacity needed to create severe and moderate water stress conditions

Water stress treatments	Fine textured soils (clayey)	Course textured soils (sandy)
	Field capacity level	Field capacity level
• Severe water stress	20%	30%
• Moderate water stress	60%	60%
• Well-watered	100%	100%

Continuing with our example the respective amounts of water needed to bring the air dry soil in pots to severe and moderate moisture stress conditions is indicated in Table 2. The soil in our example was a sandy soil, hence the use of 30% field capacity moisture and not 20%. Notice that the calculated figures have been rounded off for easier measuring. The mass equivalents of the volume of water to be added have also been calculated and included in Table 2. You will however be adding the water to the soil in volumes to bring the soil to a certain mass. For this you will need measuring cylinders of at least three different sizes. Larger measuring cylinders can be used to add greater volumes of water to the soil, particularly at the start of the experiment, but small measuring cylinders are needed for adding volumes that are less than 10 ml.

Table 2: Amount of water needed to bring soils in pots to severe and moderate water stress conditions

Water stress treatments	Required Field capacity level	Volume of water to add to soil	Approximate volume	Approximate mass
		(ml)	(ml)	(g)
• Severe water stress	30%	277.8	278	278
• Moderate water stress	60%	555.6	556	556
• Well-watered	100%	926.0	926	926

Using the amount of water needed to bring the oven dry soil to field capacity, we can now calculate the mass that our 5000 g of oven dry soil will have at field capacity. The equation below shows how this can be calculated. Remember that each pot is filled with an equivalent amount air dry soil that is needed to give 5000 g of oven dry soil. From the equation it can be seen that our potting soil will have a mass of 5933.5 g at 100% field capacity. Using the same equation we can also calculate the respective mass of the potting soil under severe and moderate water stress. Table 3 shows the respective masses of the potting soil at severe and moderate water stress and under well-watered conditions. From Table 3 it can be seen that the mass of soil in the pots under severe stress, moderate stress and well-water conditions will respectively be 5285.5 g, 5563.5 g and 5933.5 g. This value does not however consider the mass of the pot nor of the plant. Our calculations will thus continue.

$$\begin{aligned}&\textbf{mass of 5000 g of oven dry soil at field capacity}\\&= \textbf{mass of air dry soil} + \textbf{mass of water needed to bring soil to field capacity}\\&\qquad\qquad = \mathbf{5007.5\,g + 926\,g}\\&\qquad\qquad = \mathbf{5933.5\,g}\end{aligned}$$

Table 3: Mass of potting soil under severe and moderate water stress conditions and under well-watered conditions

Water stress treatments	Field capacity level	Mass of water added	Mass of moist potting soil
		(g)	(g)
• Severe water stress	30%	278	5285.5
• Moderate water stress	60%	556	5563.5
• Well-watered	100%	926	5933.5

6 **Weighing soil filled pots**

It is important for us to also talk about the weighing instruments. This is because weighing and thus weighing instruments are critical instruments for making water stress treatments. Weighing instruments need to be tested to ensure that they produce the needed results before the experiment is begun. Testing should not be left to a later time as this could ruin the entire experiment. Much planning should also be given to selecting the type of weighing instrument to be used. As some screen houses may not have a power supply, an electronic digital balance may not be suitable and even if there is a power supply you would still need to ensure that the instrument can be safe in the screen house as moving these instruments to the screen house every day is not advisable. A battery run balance could be used but make sure that you consistently have a good supply of strong batteries as weak batteries may lower the instruments sensitivity, which could ruin your experiment. A hanging scale would also not be ideal as a weighing instrument. A simple portable mechanical kitchen weighing scale would however be ideal for the job (Fig 5).

Figure 5. Mechanical portable kitchen weighing scale

A mechanical kitchen weighing scale is a simple instrument that does not need to be powered and can be easily moved around from one point of the greenhouse to the next as you weigh all the pots in the experiment. This light scale also helps you save energy as pots can be heavy to lift and carry around. This scale also costs less than other fancy weighing machines, making water stress experiments affordable for everyone. However, you will have to ensure that the scale can weigh weights of at least 10 kg or any other weight you will be working with. The scale in our example has a 20 kg weight capacity and can thus weigh our 5 kg to 6 kg of dry and wet soil without any problem. It however weighs to the nearest 100 g (Fig 6), but you can still estimate the reading to the nearest 1 g. A weighing instrument that can weigh to the nearest ± 1 g is however more ideal. Kitchen scales with a lower capacity like 10 kg and 5 kg scales would be able to weigh to the nearest ± 50 g or ± 25 g, respectively. They should be used for their better accuracy if your pot weights are low. Make sure you learn how to use the scale properly and do not forget to 'zero' or 'tare' the machine with the weight of the steel bowl before placing your pots on it. This will help you to determine the mass of the pots alone without the inclusion of the mass of the steel bowl on the weighing scale.

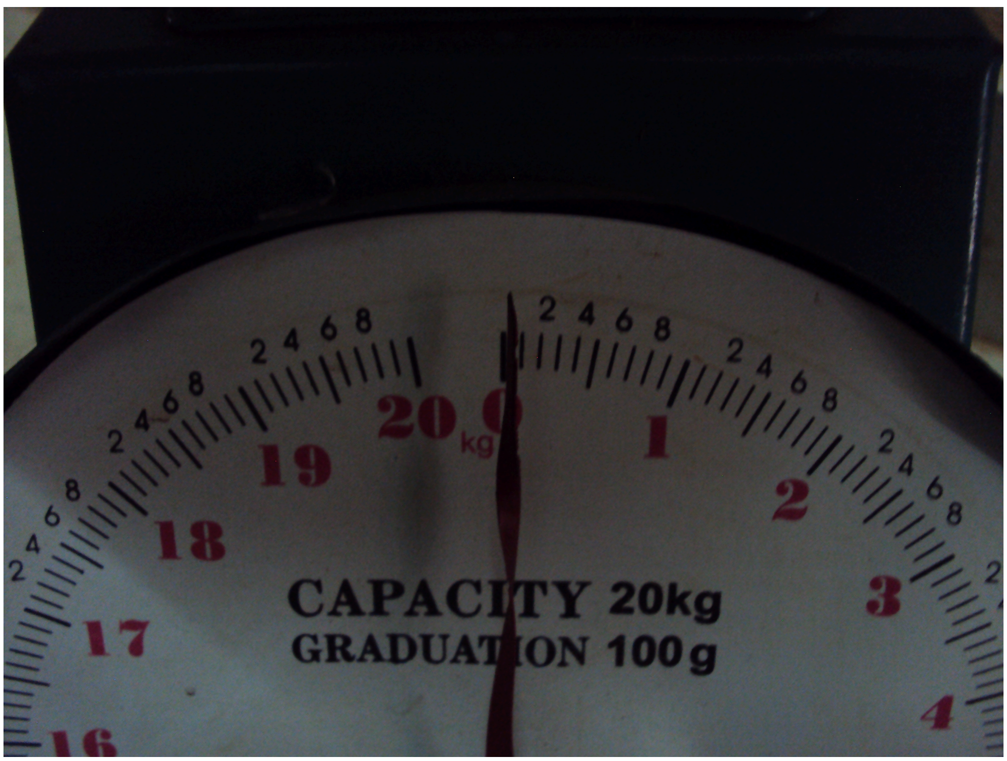

Figure 6. A close view of graduations on the weighing scale

7 **Factoring in the mass of the pot**

We now know the mass of moist soil under severe and moderate water stress and also under well-watered conditions. The moist soil mass will however be in a pot and will have to be weighed together with the pot. The mass of the pot will thus have to be accounted for by adding its mass to the mass of the air dry soil. Like the oven dry soil, the mass of the pot is a consistent mass that is never changing. Only the mass of the moisture content of the soil and to a certain extent the mass of the growing plant in each pot (to be discussed in next Step) are continuously changing. The mass of the pot must be added to the mass of air dry soil. To make pot mass measurements easier it is best to use pots with a uniform mass. Plastic pots of the same capacity and brand, usually have approximately the same mass (Fig 7). In our example the mass of the pots used was approximately 210 g. Table 4 shows the mass of the pot plus the respective mass of moist soil under severe and moderate water stress and well-watered conditions. The rounded off mass is easier to use when bringing pots to their required soil moisture contents. Always round off towards the end of all your calculations as this reduces error.

Figure 7. Plastic pots of the same capacity waiting to be filled with potting soil

Table 4: Mass of pot plus moist soil under severe and moderate water stress conditions and under well-watered conditions

Water stress treatments	Field capacity level	Mass of pot	Mass of moist potting soil	Mass of moist potting soil + pot	Approximate mass
		(g)	(g)	(g)	(g)
• Severe water stress	30%	210	5285.5	5495.5	5496
• Moderate water stress	60%	210	5563.5	5773.5	5774
• Well-watered	100%	210	5933.5	6143.5	6144

8 **Factoring in the mass of the plant**

In Step 7 we only accounted for the mass of the pot and we left out the mass of the plant. Let us first look at the equation below to get a better understanding of all the masses that must be considered before determining the mass of water needed to bring the soil to its required moisture content.

$$\text{total mass of pot (g)} = (\text{mass of pot} + \text{mass of plant} + \text{mass of oven dry soil}) + \text{mass of soil moisture}$$

A simplified version of the equation is given below:

$$\text{total mass of pot (g)} = (\text{pot} + \text{plant} + \text{soil}) + \text{soil moisture}$$

soil moisture mass is constantly changing with evapotranspiration

mass in brackets is constant, but note that the mass of the plant needs to be periodically reviewed as the plant grows

At the beginning of the experiment the weight of the plant is usually negligible as it may only be a seed or seedling at this time. Due to this, the mass of the plant can be ignored at planting and during the first weeks after planting. Most experiments usually also have all pots well-watered before beginning the moisture stress treatments. Weighing may thus not really be necessary before beginning the water stress treatments. The pots are kept well-watered simply by using visual judgement during watering. If you however decide to weigh the pots to accurately bring them to 100% field capacity every day, this would be more ideal. Note that weighing every pot in the entire experiment is no small task and needs serious commitment. The soil in the pots can be brought to 100% field capacity everyday using the equation below. Note that the same equation can be used to calculate the amount of water needed to bring the soil to 30% or 60% field capacity, when the water stress treatments are begun.

$$\text{mass of water needed to be added (g)} = (\text{total mass of pot at X\% field } capacity) - \text{curent total mass of pot}$$

$$where;\ X\%\ is\ 30\%, 60\%\ or\ 100\%\ field\ capacity$$

Note: Remember to convert the mass of water obtained to its equivalent volume, as water is added to pots in volumes, although calculated as a mass (see calculation in Step 4). The equation above assumes that the mass of the plant is zero, as it is negligible.

Once it is time to start the water stress treatments, it will be important for you to know the mass of the plant so that you can account for it. Note that the procedure to determine the plant mass at this time is the same procedure that can be used to determine the initial mass of the plant at planting or at any other time.

Determining the weight of the plant is actually tricky to do. Try to imagine how it can be done before reading the method described below:

You will firstly need to set aside extra replicates of your treatments for this purpose. These replicates should be treated the same as the plants in the pots from which you will collect your experimental data. Label these pots clearly using unique identification. Set aside at least three replicates for each treatment. More replicates would actually be better but this would make the experiment unmanageable or too expensive for certain kinds of experiments. You will need to determine the mass of the plant for each treatment a day before beginning the water stress treatments (or a day before continuing the water stress treatments once they have begun when reviewing the mass of the plant). In my experiment it was at 70 days after planting. At this time, the nutrient treatments that had been applied had taken effect and some plants were now visibly bigger than others. Note that if no treatments have been applied to the pots yet, you will still need to determine the mass of the plant so that you can account for it. If this is the case there would only be one treatment at this time and the mass of the plant to be determined will represent the mass of all plants in the experiment at this point in the experiment. Now, follow the steps below to determine the mass of the plant for each treatment.

1. Take one pot of each treatment from the pots set aside for determining plant mass.

Note: Remember that using at least two or three pots would be better for replication of results. One pot is however good enough as you are only trying to get a representative mass of the plant for that particular treatment. Note that you do not need to use all the pots that have been set aside for this purpose at this time, as some pots must be spared for the determination of plant mass at another period of time when reviewing the mass of the plant. Note that the plant is continuously growing and changing its mass and that because of this the mass of the plant must be periodically reviewed and the total mass of the pots changed accordingly to accurately bring the pots to their required moisture contents. I advise that the mass of the plant must be reviewed weekly. The number of pots set aside must thus fit the number of weeks in the water stress period. Hence, if the plants will be stressed for 20 days, then the mass of the plant for each treatment will be determined twice during this period. Using this example, the minimum number of pots to set aside for each treatment, would be at least two.

2. Weigh the pot and determine the amount of water needed to bring it to 100% field capacity. Add this amount of water to the pot.

3. Now start adding extra 100 ml volumes of water to the pot every 3 to 5 minutes, while continuously watching out for the commencement of leaking. Stop adding the extra 100 ml volumes of water once leaking begins. At this point the soil is completely saturated. This step is best carried out in the early evening when ambient temperatures are cool and evapotranspiration is low.

4. Leave the pot to stand for about 30 minutes or until a time when you are sure that the pot has stopped leaking. At this point the soil will be at field capacity.

5. Quickly weigh the pot and record its mass.

6. You can now calculate the weight of the plant by subtracting the initial mass of the plant plus pot and soil at field capacity (calculated before beginning the experiment) from the current mass. See the equation below to better understand the calculation to be carried out.

$$\text{mass of plant} = (\text{total mass of pot at field capacity})t_X - (\text{total mass of pot at field capacity})t_0$$

where; t_X = current time period and t_0 = at planting time

Alternatively;

$$\begin{aligned}&\text{mass of plant}\\&= (\text{mass of pot} + \text{mass ofoven dry soil} + \text{mass of soil moisture at 100\% field capacity} + \text{mass of Plant } t_x)\\&- (\text{mass of pot} + \text{mass ofoven dry soil} + \text{mass of soil moisture at 100\% field capacity} + \text{mass of Plant } t_0)\end{aligned}$$

The equation all comes to this;

$$\begin{aligned}&\text{mass of plant}\\&= (\text{pot} + \text{oven dry soil} + \text{soil moisture at 100\% field capacity} + \text{Plant } t_x) - (\text{pot} + \text{oven dry soil}\\&+ \text{soil moisture at 100\% field capacity} + \text{Plant } t_0)\end{aligned}$$

$$\begin{aligned}=>\ &\text{mass of plant}\\&= (\cancel{\text{pot} + \text{oven dry soil} + \text{soil moisture at 100\% field capacity}}) - (\cancel{\text{pot} + \text{oven dry soil}}\\&\cancel{+ \text{soil moisture at 100\% field capacity}}) + \text{Plant } t_x - \text{Plant } t_0\end{aligned}$$

$$=> \quad \text{mass of plant (g)} = \text{Plant } t_x - \text{Plant } t_0$$

Note that after complete wetting the mass of wet soil together with the mass of the pot will be just as they were at planting i.e. at time zero $\mathbf{t_0}$. We also know the initial mass of the plant at planting, which we assumed to be negligible and thus zero. Only the mass of the plant will be different now as the plant has grown bigger. The soil is brought to field capacity so as to leave only one unknown variable mass i.e. the current mass of the plant at time $\mathbf{t_X}$. Remember that this equation can be used to determine the mass of the plant at any time during the experiment.

You can now include the calculated mass of the plant to the respective treatment pots and determine the water to be added to bring them to 30%, 60% or 100% field capacity. **Note** that when starting the water stress treatments the pots that will be severely and moderately stressed should not be watered until the moisture in the pots has dropped to a point slightly below 30% and 60% field capacity respectively. Thereafter, the soil moisture content must be maintained at these levels in these pots following all the above calculations. The weight of the plant must thus be determined when the plants are about to reach the moisture levels below 30% and 60% field capacity. Thereafter the moisture content should be corrected daily and brought to 30%, 60% and 100% field capacity in respective pots.

9 **Some important things to note**

Notice that it is always essential to bring the pot to field capacity, before determining the mass of the plant in each pot. It is also important for you to know that once a pot has been used to determine the mass of a plant, it cannot be used for this purpose again. This is because bringing the pot to field capacity will destroy the uniformity of treatments given to plants under the same treatment. For example imagine if the plant was under severe stress. Bringing the soil of this plant to field capacity would destroy the effects of severe water stress, this is why it cannot be used again. So remember to completely separate pots that have already been used to determine plant mass from the other pots or simply empty out the pots once they have been used. Also note that treatment pots that have been set aside for plant mass determination must be treated exactly in the same way as plants in the actual experiment until after you have determined the plant mass from these pots.

Before beginning the experiment, the air dry soil in pots must be watered to field capacity one day after planting to create conducive planting conditions. As you will already know the mass of air dry soil in the pots, then you should simply add the volume of water needed to bring the soil to field capacity.

Note that the demand for watering is low in the beginning of the experiment. This is because of the low evapotranspiration rates in the pots when the plants are still young. This however changes as the plant grows.
Remember to label your pots very well so that you do not mix-up your treatments. As a demonstration of the results of this protocol see Fig 8. In the figure you will see unfertilised cassava plants that have been severely and moderately moisture stressed as well as well-watered. Notice that the amount of foliage visibly reduces with water stress.

Figure 8. Unfertilised plants under severe water stress (left), moderate water stress (middle) and under well-watered conditions (right)

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