Study Of the Effect of MS, DKW, And Wpm Media on *In-Vitro* Micropropagation of Ginger

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Abstract:
This study aims to determine the most suitable culture media for growing ginger plantlets in a controlled environment. Three different types of growth media Murashige & Skoog (MS), Driver and Kuniyuki Walnut (DKW), and Woody Plant Medium (WPM) were analyzed to identify the most optimal one that promotes the best growth of Ginger (*Zingiber officinale* Rosc.) plantlets. Ginger plantlets were inoculated into each medium and incubated under specific light and temperature conditions to assess their performance. The DKW Media supported the highest fresh weight mean of 753.33 mg. The mean dry weight was 129.2 mg. Conversely, The MS medium proved the most effective for shoot initiation, averaging 8 days and the MS medium excelled in root initiation, averaging 10 days. MS medium also resulted in the maximum plant height, averaging 4.53 cm. The dry weight percentage in the MS medium averaged 86.2%. DKW medium was found to be the most effective for fresh weight and dry weight. However, MS medium performed better for shoot initiation, root initiation, plant height, and dry weight percentage. Through this study, we have tried to develop the best culture conditions for producing ginger plantlets which can help to improve the creation of more effective *in-vitro* propagation techniques and ultimately increased production of ginger.

Keywords: MS (Murashige & Skoog), DKW (Driver and Kuniyuki Walnut), WPM (Woody Plant Medium), Invitro, Micropropagation.

I. INTRODUCTION

Ginger belongs to the family Zingiberaceae. (Nafiseh Shokri Mashhadi, 2013) This family of plants, which refers to the Zingiberales order, is made up of aromatic, herbaceous herbs that are frequently cultivated from rhizomes every year. Generally, they grow well in tropical and subtropical climates, having flower on top of the leaf (Estouka et al., 2021). Literature shows that ginger may have been introduced to Pakistan and and initially cultivated there beginning in the 13th century. Despite the unfavorable environment of Pakistan, it is regularly produced there as well (Sabir & Malik, 2023). It's a great addition to diets because it contains bioactive chemicals that have anti-inflammatory, antioxidant, and anticancer properties .Ginger's demand in global marketplaces has significantly expanded due to its numerous distinctive properties. The ginger plant is infertile and has not produced seeds because its rhizomes are employed for vegetative growth, it is therefore incapable of being sexually reproduced (Nair, 2019).It is a significant economic crop valued for its fragrant rhizomes, is a member of the *Zingiberaceae* family. Because of its many applications, it is a big cash crop that helps producers make money to survive and is in great supply internationally (Sodangi, 2021). Ginger is a slender perennial which reaches 60 to 100 cm in height (Govindarajan, 1983). Ginger rhizomes are used to make a variety of products, including as dried, powdered, fresh, and oil Ginger is grown all over the world, including India, China, Indonesia, Nepal, Thailand, Nigeria, Bangladesh, Japan, and the Philippines ranking as the top producers (Vasala, 2012). When conventional methods are not feasible for large-scale multiplication, plant micropropagtion, growing plant cells or tissues in a synthetic nutritional solution, the primary concern here is preventing *in-vitro*microbial contamination (Sodangi, (2020). Ginger can only be propagated vegetatively and is sterile in reproduction (Ravindran and Nirmal Babu 2005). To improve the commercial cultivation of virus-free ginger, *in-vitro*induction of micro rhizomes in ginger can produce disease-free plantlets. (Uozumi et al. (1994)) proposed *in-vitro*propagation for ginger seed production, but these methods are ineffective, expensive, and inappropriate for the conservation of ginger germplasm. MS is the most often used base media for plant tissue culture (Murashige and Skoog (1962). And their adaptations, such half-strength MS (½-MS); 82% of the 5-year collection of citations employed MS-based media (Herman 2015). Although at some time in the past, B5 medium or its variations were frequently employed, in this 5-y set of citations, B5-related media were utilized in only 5% of the cases. (Gamborg et al. (1968)). Woody plant basal media, like Driver and Kuniyuki and (Lloyd and McCown's (1980)) Woody Plant Medium (WPM) Six percent of the 5-

year collection of citations employed woody plant media (DKW; Driver and Kuniyuki 1984). An additional 7% of the citations utilized ½-MS or MMS (modified MS; ½-macro MS salts + full-strength micro MS salts + B5 vitamins) for woody plant applications. Six percent more citations used half strength MS for rooted applications (Gamborg et al. (1976)). Woody plants have lower levels of nitrogen and ammonium, which lowers the danger of ammonium toxicity, they frequently develop on medium like ½-MS, MMS, and WPM. On the other hand, DKW medium contains more sulfate than MS, which may be beneficial for some woody plants even if it has a same ammonium ratio. (Gregory C. Phillips1, 2019) . In this study, we evaluated the effects of three different tissue culture media (MS, DKW, and WPM) on the in-vitro growth characteristics of ginger. We measured parameters such as shoot and root initiation, plant height, and biomass to determine which media supports optimal growth. In this study, we evaluated the effects of three different tissue culture media (MS, DKW, and WPM) on the in-vitro growth characteristics of ginger (*Zingiber officinale Rosc*.). We measured parameters such as shoot and root initiation, plant height, and biomass to determine which media supports optimal growth.

II. MATERIALS AND METHODS

The experiment "**Study of the effect of MS, DKW, and WPM media on *in-vitro* micro propagation of ginger***"* was carried out at Agricultural Research Institute (ARI), Tarnab, Peshawar, Pakistan, during October 2023 to January 2024.

3.1. Plant Material

Fresh and healthy *in-vitro*plantlets of ginger developed at Plant Tissue Culture Laboratory, ARI, Tarnab, Peshawar, were evaluated for the current study.

3.2. Media selection

Composition	DKW	MS	WPM
NH4NO3	17.600	1650	400
H3BO3	0.078	6.2	6.2
CaCl2	1.01	332.2	72.47
CoCl2.6H2O	0	0.025	0
CuSO4.5H20	0	0.025	0
C10H12N2NaFeO8.3H20	120	37.26	37.3
FeSO4.7H2O	0	27.8	27.85
MgSo4	3.000	180.7	180.7
Mnso4.H2O	0.200	16.9	22.3
Na2MoO4.2H2O	0.002	0.25	0.25
Ki	0	0.83	0
KNo3	0	1900	0
KH2PO4	1.950	170	0
ZnSO4.7H2O	0.072	8.6	8.6
Ca(NO3)2.4H2O	8.300	0	556
CuSo4	0	0	0.16
K2SO4	8.950	0	990

Three Medias i.e. MS, DKW and WPM. The MS media containing micro and macronutrients, Vitamins, Sucrose 3 %, Growth regulators such as Auxins and cytokinins, for roots and shoot initiations Agar 7 g/l, and pH 5.8 were maintained throughout the research study. Before autoclaving at 121 °C for 20 min, the test tubes were filled with 8 ± 1 ml media and sealed with cotton plugs.

Fig. 1 Pictorial presentation of media preparation (*A-preparing glassware, B-weighing MS media and sucrose, C-pH adjustment of media, D- pouring media in test tubes after dissolving agar, E-autoclaving media*

3.2. Treatments. When the three media namely MS, DKW, and WPM were applied, resulted the following treatments.

Table 1: Treatment such as T1 for MS, T2 for DKW and T3 for WPM Medias

Treatments	Media type	Composition
T1	MS (Murashige & Skoog)	NH4NO3, H3BO3, CaCl2, C10H14N2NaFeO8.3H2O, FeSO4.7H2O,MgSO4, MnSO4.H2O, KI, KNO3, KH2PO4, ZnSO4.7H2O
T2	DKW (driver and Kuniyuki walnut)	NH4NO3, H3BO3, CaCl2, C10H14N2NaFeO8.3H2O, MgSO4, MnSO4.H2O, KH2PO4, ZnSO4.7H2O.,Ca(NO3)2.4H2O,K2SO4
T3	WPM (woody plant medium)	NH4NO3, H3BO3, CaCl2, C10H14N2NaFeO8.3H2O, MgSO4, MnSO4.H2O, ZnSO4.7H20,Ca(NO3)2.4H2O ,CuSO4, K2SO4

3.3. Performing the experiment

3.3.1 Inoculation

The already grown plantlets were taken, cut and inoculated in the desired media. The plantlets were typically sub cultured in a test tube using a single piece of cut plantlet, and the process was carried out inside the laminar flow hood using a sterile scalpel, surgical blade, and Petri plates.

Fig. 2 Pictorial presentation of inoculation of callus. ***A-explant (invitro grown plants used for the experiment) B-explant preparation), C-culturing the plantlets in the desired media, D- sealing the test culture tube with cotton plug.***

3.4. Incubation

After inoculation, the cultures were incubated in the growth chamber with temperature (25 ± 1 °C), relative humidity (70%), light intensity (40–50µ mol/m2/s), and photoperiod of 16 h light and 8 hs dark. For each treatments were done in replicates, and recorded.

Fig. 3 Incubation of culture in Growth chamber.

3.5 Root initiation media:

Rooting was evaluated as a function of media, namely MS, DKW, and WPM all supplemented with auxins and adjusted to pH 5.7 - 5.8. Prior to the experiment, plantlets were placed in a culture bottle under aseptic conditions and incubated

within 25 ± 1 °C, at 70% of relative humidity with a 16 h photoperiod. The development of roots was measured in course of time in different treatments in order to assess the effectiveness of the media.

3.6 Shoot initiation media:
Shoot initiation was done using MS, DKW, and WPM media which included cytokinins to promote the shoot growth. Under sterile conditions, ginger plantlets were immersed in the media and subsequently put in an incubator at 25 ± 1°C, 70% humidity, and 16 hours' light duration. Shoot formation was noted and recorded to assess the efficacy of the media used.

III.RESULTS
The applied treatments i.e. T1, T2, and T3 were screened for the suitable physical growth characteristics of Ginger.

3.5.1. Plant Morphology
This includes different aspects of the plant morphological characters as mentioned below.

3.5.1.1. Days to shoot initiation:
The Shoot initiation for R1 takes 10 days under DKW conditions (T1), 8 days under MS conditions (T2), and 13 days under WPM conditions (T3). This indicates that shoot initiation occurs more rapidly in MS media as compared to DKW media and WPM media.

Table (1) Effect of different Medias (MS, DKW, WPM) on shoot initiation

	MS	DKW	WPM
	T1	T2	T3
(Replication 1)R1	8 days	10 days	13 days
(Replication2)R2	7 days	9 days	12 days
(Replication 3)R3	9 days	11 days	14 days
Average	8 days	10 days	13 days

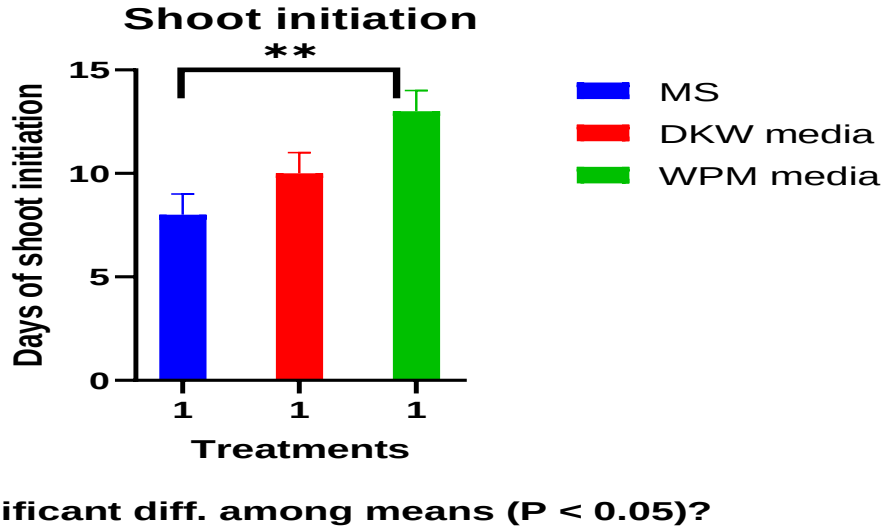

Figure 1. Effect of different Medias (MS, DKW, WPM) on shoot initiation. Control T1, T2, T3 (P<0.05)

3.5.1.2. Days to root initiation
The root Initiation of Ginger for R1 MS media (mean of 11 days), DKW media (mean of 14 days), and WPM media (mean of 18 days). This suggests that MS media is the most effective in starting a shoot as compared to DKW (T2) and WPM (T3) conditions.

Table (2) Effect of different Medias (MS, DKW, WPM) on root initiation.

	MS	DKW	WPM
	T1	T2	T3
R1	12 days	16 days	17 days
R2	10 days	14 days	19 days
R3	11 days	13days	18 days
Average	11 days	14 days	18 days

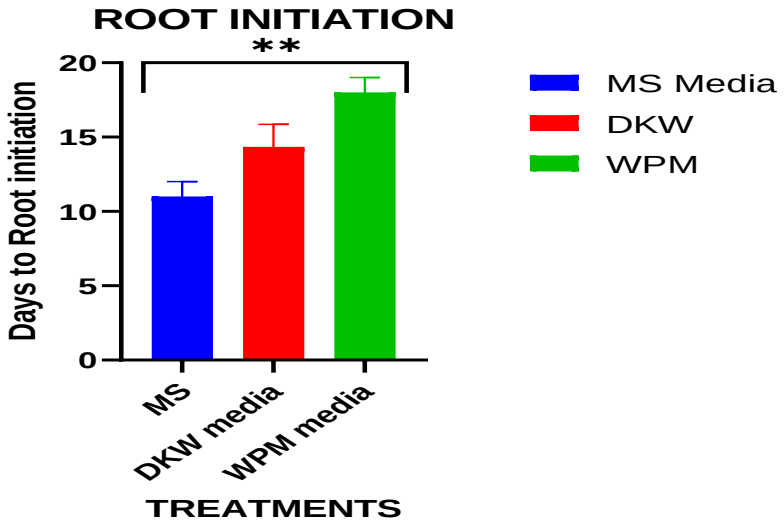

Figure (2) Effect of different Medias (MS, DKW, WPM) on root initiation. (P<0.05)

3.5.1.3. Plant height

Ginger plant height for R1 is seen under MS media (Average of 4.53cm), DKW media (Average of 4cm), and WPM media (Average of 3.03cm). This implies that MS media indicate most effective outcome followed by DKW and WPM media.

Table (3) Effect of different plant Medias (MS, DKW, WPM) on height of plants in (cm)

	MS	DKW	WPM
	T1	T2	T3
R1	4.5cm	4cm	3cm
R2	4.7 cm	3.8 cm	3.2 cm
R3	4.4 cm	4.2 cm	2.9 cm
Average	4.5 cm	4 cm	3 cm

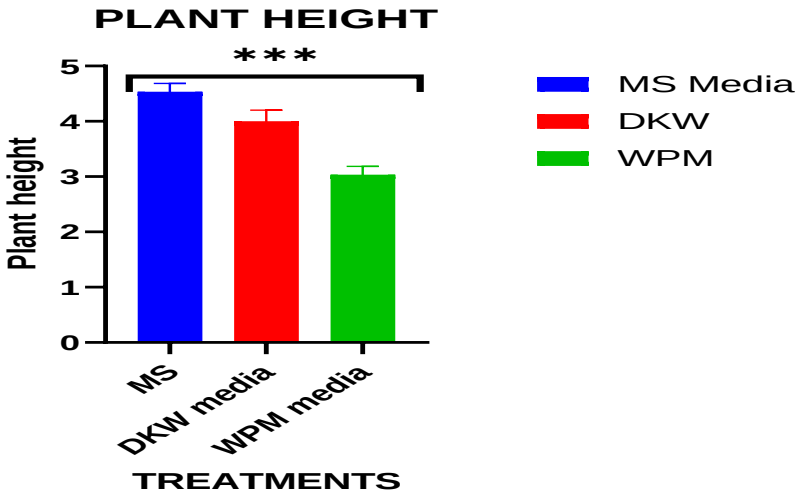

Figure (3): Effect of different plant Medias (MS, DKW, WPM) on height of plants in (cm). (P<0.05)

3.5.2. Plant biomass

This includes the fresh weight, dry weigh, and percent moisture content.

3.5.2.1. Fresh weight

Three distinct media types are used to measure the fresh weight of ginger for R1 at different times: MS media (mean of 606.66 mg), DKW media (mean of 753 mg), and WPM media (mean of 551.66). Generally, it seems that plants treated with DKW have larger fresh weights than plants treated with MS and WPM.

Table (4): Effect of different plant Medias (MS, DKW, WPM) on fresh weight of plants in (mg).

	MS	DKW	WPM
	T1	T2	T3
R1	600	750	550
R2	590	720	535
R3	630	790	570
Average	606	753	551

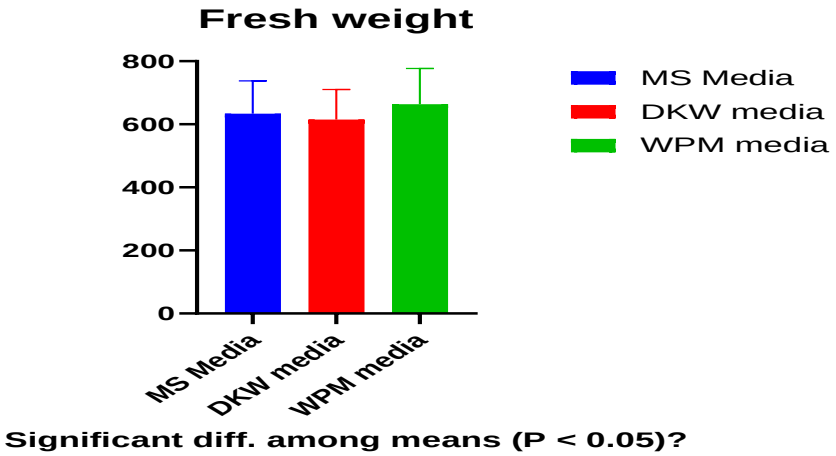

Figure (4): Effect of different plant Medias (MS, DKW, WPM) on fresh weight of plants in (mg).

3.5.2.2. Dry weight

Dry weight for R1 show best result under DKW media (Average of 129.197 mg), MS media (Average of 83.62 mg), and WPM media (Average of 88.5). When compared to MS and WPM media, DKW media regularly displays the greatest outcome, having the highest mean value of 129.20mg.

Table (5): Effect of different plant Medias (MS, DKW, WPM) on Dry weight of plants in (mg).

	MS	DKW	WPM
	T1	T2	T3
R1	83.28	134.10	87.34
R2	87.14	120.53	95.44
R3	80.45	132.96	82.72

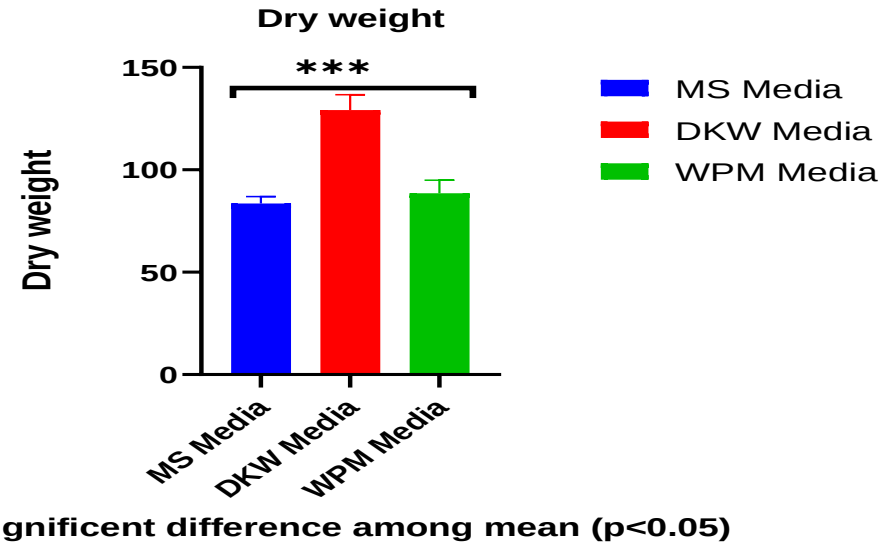

Figure (5): Effect of different plant Medias (MS, DKW, WPM) on Dry weight of plants in (mg).

3.5.2.3. Percent moisture content

According to the results, WPM media had the greatest average Moist content (83.35%), followed by DKW media (82.15%) and MS media (86.19%). When compared to DKW and WPM media, MS media provided the greatest results overall, having the highest mean percent moist content.

Table (6): Effect of different plant Medias (MS, DKW, WPM) on percent moist content in (percentage).

	MS	DKW	WPM
	T1	T2	T3
R1	86.12	82.12	84.12
R2	85.23	83.26	82.16
R3	87.23	83.17	83.78

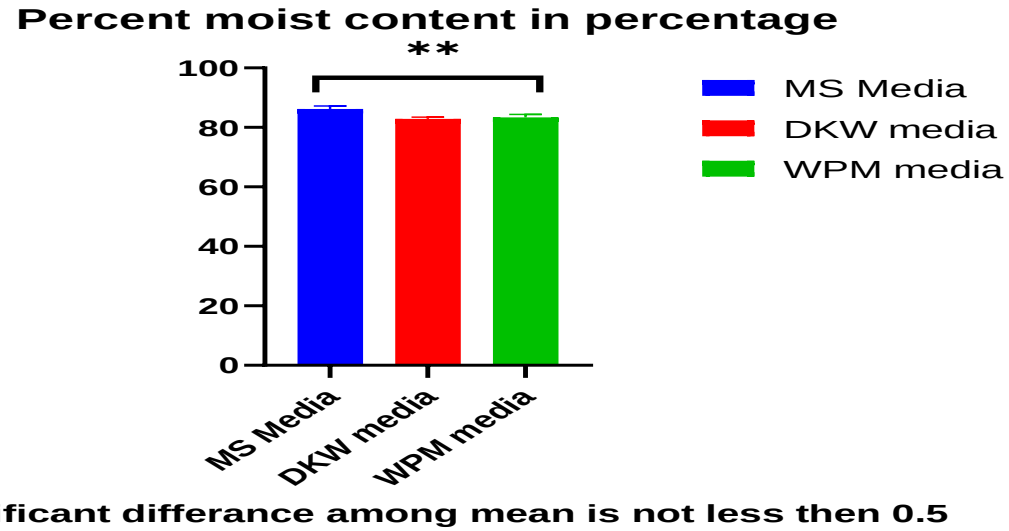

Figure (6): Effect of different plant Medias (MS, DKW, WPM) on percent moist content in (percentage).

IV. Discussion

The study compared three media (MS, DKW, and WPM) for ginger shoot initiation. MS medium was found to be the most effective, with fastest shoot initiation time compared to DKW and WPM. This aligns with findings by (G. R. Rout, 2001) who observed similar results using different growth regulators. Ginger meristem production occurs mostly in MS media by using different growth regulators in different concentrations such as Adenine sulphate, BA, within 28 days and this can result in up to 32.4 shoots per culture. However, broader research which was performed by(Higgins S1, 2020) shows that media fortified with specific hormones can significantly enhance shoot proliferation, as seen in studies where certain media like Linsmaier and Skoog's Basal Medium (LSBM) with BAP produced up to 19 shoots per buds.

The study found that the MS medium was the most effective for root initiation in ginger micropropagation, taking a short time compared to DKW and WPM media. This is likely due to the optimal nutrient composition and pH of the MS medium. However, the same method applied by (seran, 2013) for Rhizome buds and aerial stem explants is useful tools in micropropagation, facilitating the production of multiple shoots. Traditional ginger propagation methods are unreliable due to disease risks and limited explant availability. A faster, reliable and disease free alternative is offered by in-vitro methods

Plants of Ginger showed various heights in different growth media types and at different times. The MS medium (Murashige and Skoog) had the highest mean plant height at 4.53 cm, followed by the DKW medium (Driver and Kuniyuki Walnut) at 4.00 cm and the WPM medium (Woody Plant Medium) at 3.03 cm. These findings imply that MS medium, DKW, and WPM media were the media types that typically promoted the tallest growth. This pattern is consistent with earlier studies showing that the balanced nutrient composition and hormone levels of MS media can enable superior growth outcomes in a variety of plant species (Murashige & Skoog, 1962) (Kuniyuki, 1984).To learn more about the distinct nutritional profiles and hormonal interactions seen in each of these media types and how they affect Ginger, more research may be necessary.

The results of this investigation, which showed that ginger plantlets grown in DKW medium had the maximum fresh weight, are consistent with findings from studies (Kubota, 2001). According to their research on photoautotrophic micropropagation systems for woody plants, DKW medium is designed for woody plants and may be more suitable for ginger due to its higher nutrient content.

DKW medium is the best for increasing the weight of ginger plants. It produces plants with significantly higher dry weight compared to WPM and MS media. This implies that the unique nutrient blend of DKW medium, with greater concentrations of calcium and magnesium in particular, is especially beneficial for ginger growth, enabling the plants to

uptake more nutrients and produce more biomass. These results will help to improve the cultivation and medicinal applications of ginger (Kubota, 2001).

Although WPM media produced the highest dry weight (mg) in the present study, MS media exceeded DKW and WPM in terms of dry weight percentage (86.19%). Variations in water content can explain this seemingly contradicting finding. A similar pattern was discovered in research by (Fatima Henkrar, 2023) looking into the micro propagation of Queues species. Their research revealed that although the plantlets' fresh weight fluctuated according to the media, the dry weight percentage was constant. MS medium might make the ginger plants thicker and drier, even though they might not weigh as much as plants grown in other media. This is because MS medium might affect the water content of the plants.

- MS media is best for shoot initiation, taking only 8 days on average.
- MS media is better for root initiation, with an average of 11 days.
- MS media also results in taller plants, with an average height of 4.53 cm.
- DKW media produces plants with the highest fresh weight, averaging 753 mg.
- DKW media again shows the highest dry weight, with an average of 129.197 mg.
- MS media has the highest percent moisture content, with an average of 86.19%.

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