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Compatibility of Jatropha (Jatropha curcas L.) grafting to increase production and drought stress tolerance

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Abstract

This study investigates the compatibility of *Jatropha curcas* grafting and its impact on morphological, biochemical, and production traits under drought conditions. A split-plot design with three replications was applied, using three high-yielding scion accessions (IP-3A, PT-7, HS-49) and seven rootstock accessions (IP-3M, A-013, A-045, C-019, C-116, D-072, D-117). Evaluated parameters included morphological traits, yield components, oil content, leaf proline concentration, and graft union histology. Data were analyzed using ANOVA followed by Duncan's Multiple Range Test (5%). Grafting significantly enhanced growth and yield components compared to those of the non-grafted plants. PT-7 was identified as the most compatible and productive scion, while IP-3M was the most effective rootstock for promoting vegetative growth. Differences in leaf proline accumulation among the various graft combinations reflected variations in drought adaptation. Compatible graft combinations resulted in a narrower necrotic layer, stronger callus formation, and more rapid re-establishment of vascular connections. The PT-7/IP-3M combination demonstrates superior graft compatibility, growth performance, productivity, and drought adaptation. The identified graft combinations offer an effective strategy to enhance the productivity of drought-tolerant *Jatropha curcas* when cultivated on marginal and semi-arid lands, while simultaneously supporting a sustainable supply of biofuel feedstock amid global climate change.

Keywords: Biofuel, Drought tolerance, Graft compatibility, Jatropha curcas, Rootstock, Scion.

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1. Introduction

The increasing demand for plant oils as biofuel sources, particularly jatropha (*Jatropha curcas* L.), is driven by rising petroleum prices and the need to reduce CO_2 emissions while enhancing fuel security. Jatropha is particularly noted for its high seed oil content, which ranges from 35% to 50%, depending on the variety [1-4]. As a non-edible oilseed, jatropha does not compete with food crops, alleviating concerns related to food security and the food-versus-fuel debate [1, 3]. In addition, they are potential to reclaim wastelands that bring a positive effect on ecology and socio-economic development [1, 3, 5, 6].

Jatropha cultivation on a large scale in Indonesia began in 2005, and in its development, going through ups and downs. Some of the main causes of low success jatropha cultivation are limited quality plant materials, soil character incompatibilities with the growing requirements of the jatropha plant material available [7, 8]. To enhance the success of jatropha cultivation in Indonesia, efforts must focus on improving the quality of plant materials and ensuring that planting occurs in suitable soil conditions [9].

In Indonesia, the potential for jatropha development spans approximately 49,531.7 million hectares [10] particularly in arid and semi-arid areas where land is marginal due to limited water availability. Jatropha exhibits remarkable drought resistance, allowing it to grow in degraded lands and semi-arid climates, which are unsuitable for food crops [11]. Therefore, jatropha development on a wide scale requires a highly productive plant material and is able to adapt to the conditions of limited water availability [12].

The superior plant material of jatropha curcas, particularly the composite selection known as IP-1 to IP-3, has shown significant potential for optimal production under favorable land conditions. Research trials have demonstrated that these superior provenances achieve the highest seed production when subjected to optimal irrigation conditions. Specifically, the highest seed yield is achieved when the soil moisture content reaches 65%, while irrigation is crucial to maintain this level of productivity. Without irrigation, seed production can decrease by up to 72.8% [13].

The stability of Jatropha production in drylands can be enhanced through advanced grafting technology, which combines high-yielding scion candidates with rootstocks that are adapted to water-limited conditions. This approach is particularly relevant given that certain Jatropha accessions have demonstrated tolerance to water shortages, making them suitable candidates for rootstock development [14].

Graft compatibility response is a complex mechanism with a wide range of different morphological, physiological, biochemical, and anatomical interactions. The compatibility response is influenced by several factors, including vascular connection, and the structural integrity of graft interfaces. Successful grafting relies on effective vascular fusion between the stock and scion. Incompatible grafts often exhibit poor vascular connections, leading to nutrient and water transport disruptions [15].

Histological studies have shown that anatomical compatibility, such as the alignment of vascular cambium, is critical for successful grafting [16]. Phytohormones, especially auxins, play a significant role in regulating graft union formation, influencing the success of grafting operations [17]. Differences in phenolic compounds and peroxidase levels at the graft union can serve as indicators of compatibility. These biochemical markers reflect the physiological responses of the graft partners [15].

The understanding of graft compatibility mechanisms can enhance Jatropha cultivation in rainfed regions by selecting compatible rootstock-scion combinations for improved growth and survival [17, 18]. High-yielding scions combined with drought-resistant rootstocks significantly enhance the productivity and resilience of Jatropha in rainfed regions [19, 20].

Continued research into the specific accessions and their compatibility will be essential to optimize this approach. However, little is known about the biochemical basis for incompatibility and the morphological mechanism involved in this response. This study aims to examine the morphological, biochemical, and histological mechanisms underlying graft compatibility in physic nut, specifically the grafting characteristics between the scion and rootstock of plants growing naturally in rain-fed agricultural areas (characterized by limited soil water availability).

2. Research Methods

The planting materials that were used included three high yield as scion (IP-3A, PT 7 and HS 49) and seven deep and spread root system as root stock (IP-3M, A-013, A-045, C-019, C-116, D-072 and D-117). This experiment was arranged using a split-plot design with three replications. The main plots consisted of grafted plants derived from three high-yielding scion accessions (IP-3A, PT-7, and HS-49). The subplots consisted of grafted plants derived from seven rootstock accessions: IP-3M, A-013, A-045, C-019, C-116, D-072, and D-117. In addition to the grafting of combination of scion/rootstock, appropriate treatments, and in order to make a comparison, other non-grafting combination or autografting scion were also planted. Experimental unit was made up of 6 grafting plants which were planted in rows on the 6 m with spacing of 1 m x 1 m.

The seven rootstocks used for planting required cuttings of 10-12 cm in size and consisted of 3 nodes each. The cuttings were treated with an aqueous solution containing 1 ml/L Confidor (Imidapride 35% E.C.) for 30 minutes For this process, the cuttings were dipped and then taken out After that, the cuttings were planted into black 2 liter plastic bags with a media of agricultural sand and compost 1: 1 was mixed.

Grafting was done at 2 months old clonal rootstock seedlings using indoor wedge grafting technique. The grafted area was covered (rolled and tied) with special plastic parafilm [19, 21]. All grafts were maintained under nursery conditions (60% shading, 27-32°C). The plants were watered daily and treated against pests and diseases according to the current agronomic practices.

At two months later, the successful grafts were transplanted into the field to determine grafting compatibility based on real conditions. In the first month, watering was done in a weekly basis and in the second month, watering was done in a biweekly basis but in the third month, it was watered only once along with a biweekly basis. Fertilizer applied included 5 tons/ha of manure and NPK fertilizer of 100 kg/ha of Urea, 100 kg/ha of SP-36 and 50 kg/ha of KCl. Other management practices including weeding, pest and disease control that respect the technical specifications relating to jatropha crops.

Data were collected every 2 weeks for plant height, stem circumference below and above graft union, branches, width of the canopy, and leaves, flowers and fruits, weight of 100 seeds, seed yield and oil percentage. Oil content was determined by soxhlet method while proline was estimated by method as described by Bates, et al. [22]. Adaptation of compatibility criteria based on growth and production attained and biochemical characteristics at each grafted pair.

The data obtained were analyzed statistically using analysis of variance (ANOVA) based on Spit Plot Design (SPD). Comparison of quantitative traits means based on Duncan Multiple Test Distance (Duncan's Multiple Range Test Range = DMRT) were performed in SAS (2001). Excel software was used for drawing of Figures.

3. Results

3.1. Effects of Grafting Combination and Non Grafting Combination on Plant Growth Traits, Production and Oil Content

The composite plant is expected to have a combination of superior characters from both rootstock and scion. The result showed that grafting can improve jatropha morphological characters, especially the character above graft union included: plant height, stem circumference, number of branches, width of canopy, number of leaves, number of bunches, number of capsules produced, weight of 100 seeds, seed yield or seed productivity and oil content as shown in Figure 1 below.

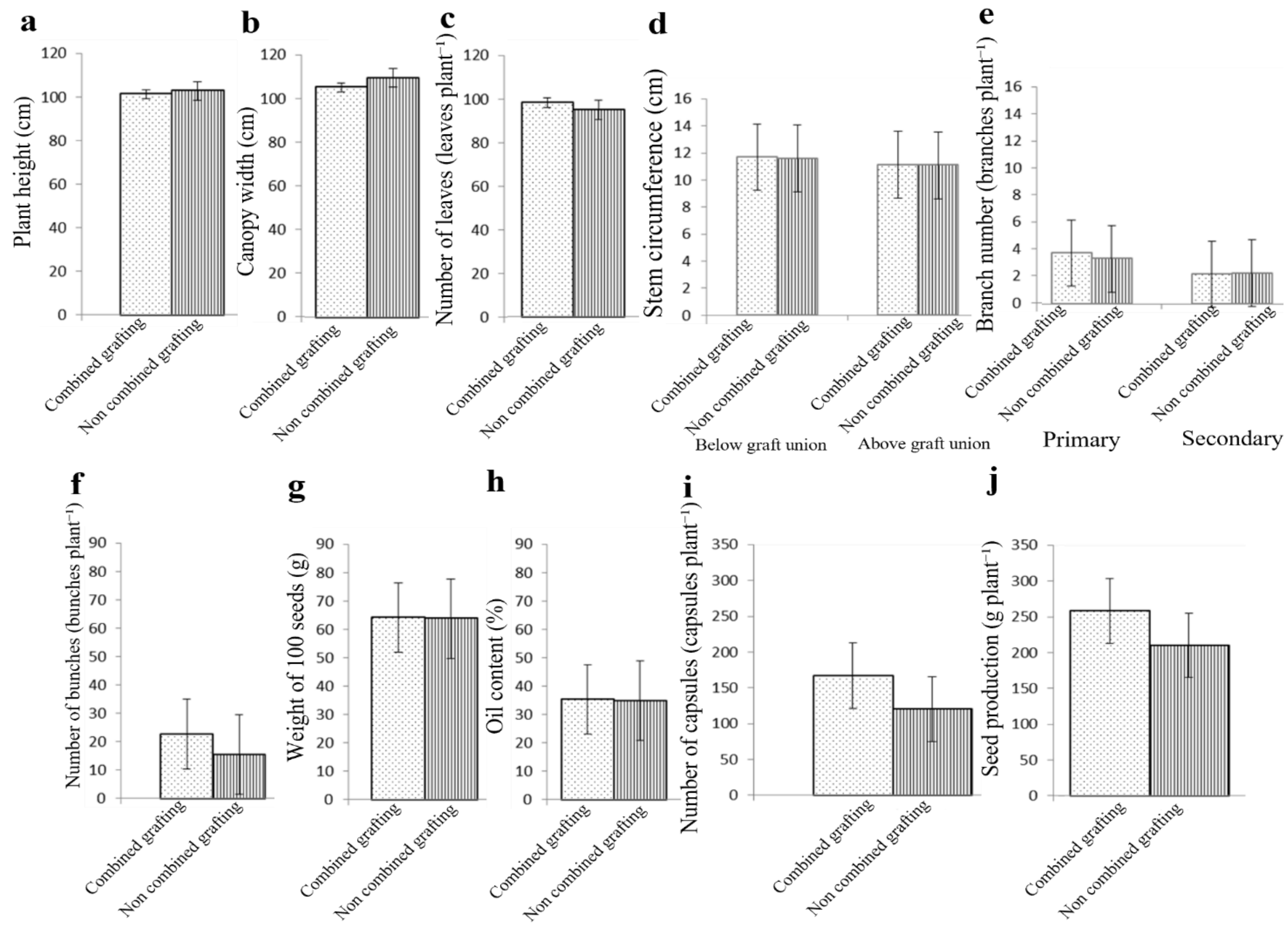

Figure 1.
Morphological traits of combined- and non-combined-grafted Jatropha plants: (a) plant height, (b) canopy width, (c) leaf number, (d) stem circumference, (e) branch number, (f) bunch number, (g) weight of 100 seeds, (h) oil content, (i) capsule number, and (j) seed production. Data are presented as mean ± standard error (SE).

3.2. Effects of Rootstock on Plant Growth Traits, Production and Oil Content of Scion

Result of Anova (analysis of variance) indicated that of rootstock accession on the morphological characters of vegetative growth, the production of components and oil content were statistically significant (Table 1 and 2). Grafting plant of different combination scions and rootstocks affected the morphological characters of jatropha plant height, stem circumference below and above the graft union, the number of primary and secondary branches, canopy width and number of leaves, number of bunches, number of capsules, weight of 100 seeds, seed production, and oil content.

Table 1.
Effect of rootstock accessions on vegetative growth of scion accessions.

Accessions		Plant height	Stem circumference below graft union	Stem circumference above graft union	The number of primary branches	The number of secondary branches	Canopy width	Number of leaves
Scion	**Rootstock**	**(cm)**	**(cm)**	**(cm)**	**(branches)**	**(branches)**	**(cm)**	**(leaves)**
IP-3A	IP-3M	104.80 a	12.35 a	11.99 a	3.78 a	2.17 a	102.61 a	93.78 a
	A-013	103.29 a	11.94 a	11.61 ab	2.99 ab	1.06 b	99.42 a	75.38 abc
	A-045	103.69 a	11.93 a	11.41 ab	2.72 ab	1.33 b	95.38 a	70.78 bc
	C-019	86.48 b	11.36 ab	10.44 ab	2.39 b	0.38 c	81.40 a	56.94 c
	C-116	102.38 a	11.67 ab	11.28 ab	2.56 ab	1.33 b	94.66 a	73.67 abc
	D-072	84.96 b	10.69 b	10.03 b	2.89 ab	1.06 b	80.33 a	58.83 bc
	D-117	95.05 ab	11.30 ab	10.67 ab	3.13 ab	2.17 a	94.34 a	80.30 ab
	-	103.47 a	11.32 ab	11.66 ab	3.22 ab	1.29 b	103.38 a	74.17 abc
HS-49	IP-3M	110.97 a	12.43 a	12.22 a	4.88 ab	2.98 a	117.63 ab	120.22 a
	A-013	107.40 ab	11.92 ab	11.57 ab	4.52 abc	2.49 a	122.99 a	115.17 a
	A-045	93.40 b	10.86 b	10.79 b	3.89 abc	2.00 a	105.98 ab	103.56 a
	C-019	92.67 b	10.89 b	10.39 b	3.00 c	1.56 a	100.81 b	84.28 a
	C-116	105.69 ab	11.83 ab	11.31 ab	3.44 bc	1.89 a	109.12 ab	98.00 a
	D-072	107.04 ab	11.65 ab	11.54 ab	5.33 a	2.87 a	120.91 ab	100.43 a
	D-117	108.96 a	11.75 ab	11.46 ab	4.59 abc	2.63 a	110.92 ab	118.12 a
	-	110.07 a	11.93 ab	11.61 ab	3.78 bc	2.43 a	117.32 ab	109.39 a
PT-7	IP-3M	121.23 a	13.22 a	12.89 a	5.28 a	4.89 a	133.43 a	151.89 a
	A-013	107.45 ab	11.76 bc	11.19 b	4.39 ab	3.17 b	117.30 b	130.67 ab
	A-045	105.82 ab	11.98 b	11.22 b	4.44 ab	3.06 b	107.97 bc	115.00 ab
	C-019	77.44 b	10.63 cd	10.34 bc	3.39 bc	2.06 b	103.72 bc	91.39 b
	C-116	107.96 ab	11.22 bcd	10.72 bcd	3.61 bc	2.28 b	106.51 bc	102.44 b
	D-072	94.87 ab	10.12 d	9.31 d	3.44 bc	1.67 b	93.29 c	85.61 b
	D-117	106.91 ab	11.82 bc	11.23 b	4.44 ab	3.06 b	114.84 b	134.11 ab
	-	94.98 ab	10.72 cd	9.99 cd	3.06 c	3.17 b	108.22 bc	100.78 b

Source: Description: The numbers in each column followed by the same letter were not significantly different by Duncan's multiple range test 5%. The symbol "-" in the rootstock column indicates non-grafted plants.

Table 2.
Effect of rootstock accession to the morphological characters of production components and oil content on the scion accessions.

Accessions		Number of bunches	Number of capsules	Seed production	Weight of 100 seeds	Oil content
Scion	Rootstock	(bunches)	(capsules)	(gr)	(gr)	(%)
IP-3A	IP-3M	25.33 ab	164.67 abc	250.77 abc	70.97 a	31.94 a
	A-013	23.67 abc	187.00 ab	300.15 a	62.57 b	34.17 a
	A-045	23.00 abc	161.33 abc	243.33 abc	62.70 b	30.93 a
	C-019	18.67 cd	146.00 abc	197.07 bc	60.93 b	29.60 a
	C-116	22.00 c	160.67 abc	255.33 abc	60.73 b	33.90 a
	D-072	14.00 d	116.67 c	180.17 c	59.83 b	34.38 a
	D-117	28.33 a	193.67 a	283.43 ab	67.07 ab	33.09 a
	-	19.00 cd	129.00 c	243.37 abc	62.23 b	33.00 a
HS-49	IP-3M	24.33 a	188.00 a	282.24 ab	60.07 ab	41.84 a
	A-013	23.33 a	173.00 ab	281.18 ab	62.27 ab	34.97 cd
	A-045	18.00 a	119.67 b	198.47 b	60.07 ab	36.50 cd
	C-019	24.67 a	185.33 a	282.17 ab	58.93 b	34.27 d
	C-116	21.00 a	153.33 ab	258.67 ab	61.93 ab	35.92 cd
	D-072	22.00 a	131.67 ab	112.98 c	70.30 a	38.26 bc
	D-117	21.67 a	172.67 ab	309.63 a	66.17 ab	40.42 ab
	-	16.67 a	144.33 ab	244.80 ab	63.27 ab	37.04 cd
PT-7	IP-3M	27.00 a	201.00 a	329.91 a	68.87 a	35.35 a
	A-013	23.00 ab	188.67 a	316.24 a	66.93 a	35.11 a
	A-045	22.33 ab	168.33 a	270.50 a	67.70 a	36.91 a
	C-019	29.00 a	197.00 a	281.30 a	72.13 a	33.30 a

	C-116	21.33 ab	177.33 a	274.83 a	62.93 a	34.93 a
	D-072	14.67 bc	98.33 b	143.83 b	52.50 a	37.96 a
	D-117	28.67 a	223.67 a	369.83 a	70.17 a	37.14 a
	-	10.67 c	86.67 b	142.33 b	65.90 a	34.67 a

Source: Description: The numbers in each column followed by the same letter were not significantly different by Duncan's multiple range test 5%. The symbol "-" in the rootstock column indicates non-grafted plants.

3.3. Effects of Scion on Plant Growth Traits, Production and Oil Content

The observations from the grafting system indicate that the PT-7 scion accession is the most suitable source of scion, followed by HS-49 and IP-3A scion accessions (Figure 2 and 3). This suitability is attributed to PT-7 promoting both robust vegetative growth and significant generative growth, resulting in high fruit production and seed yield. This result aligns with the study conducted by Gong, et al. [23] on tomato plants, which likely highlights the importance of scion compatibility and its impact on plant productivity.

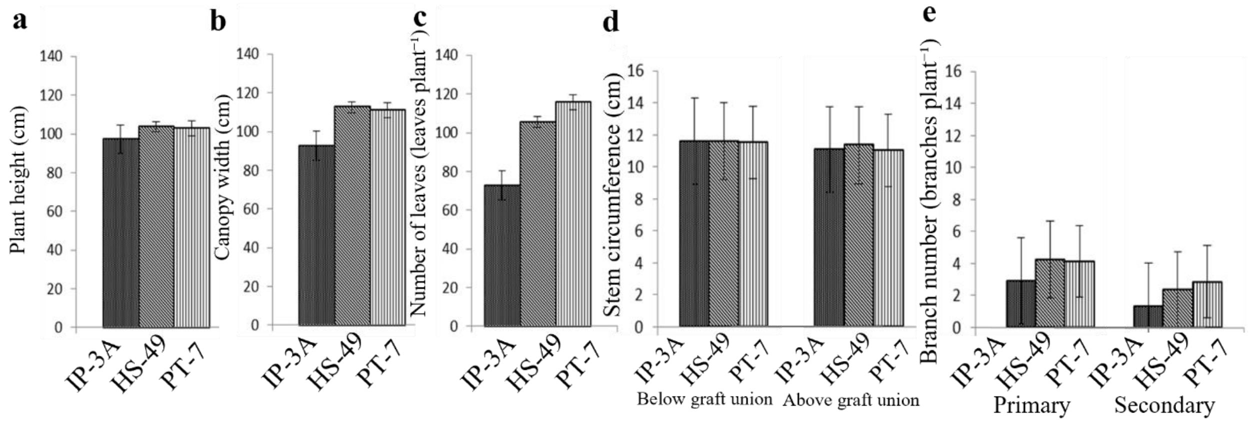

Figure 2.
Effect of scion accessions on vegetative growth traits of Jatropha plants: (a) plant height, (b) canopy width, (c) leaf number, (d) stem circumference, and (e) branch number. Data are presented as mean ± standard error (SE).

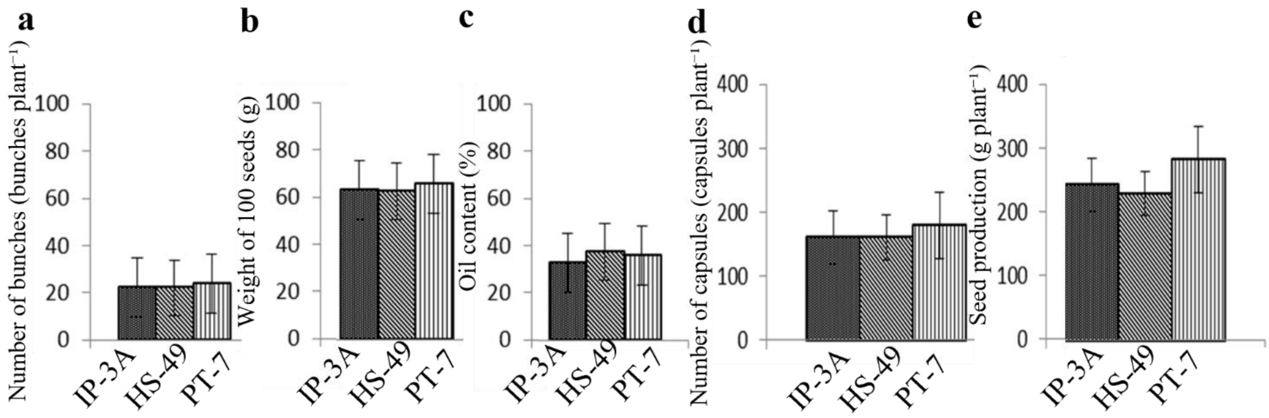

Figure 3.
The Effect of scion accessions component production and oil content: (a) bunch number, (b) weight of 100 seeds, (c) oil content, (d) capsule number, and (e) seed production. Data are presented as mean ± standard error (SE).

3.4. Effects of Rootstock on Plant Growth Traits, Production and Oil Content

The ability of two different plants to form a successful union and develop satisfactorily into one composite plant is termed graft compatibility. High graft compatibility is associated with better growth and productivity. Observations up to age 6 WAP showed that accession suitable rootstock sources such as: IP-3M, A-013, C-116 and D-117 which showed the success rate of grafting and morphological characters of growth better than other accession were tested (Figure 4 and 5).

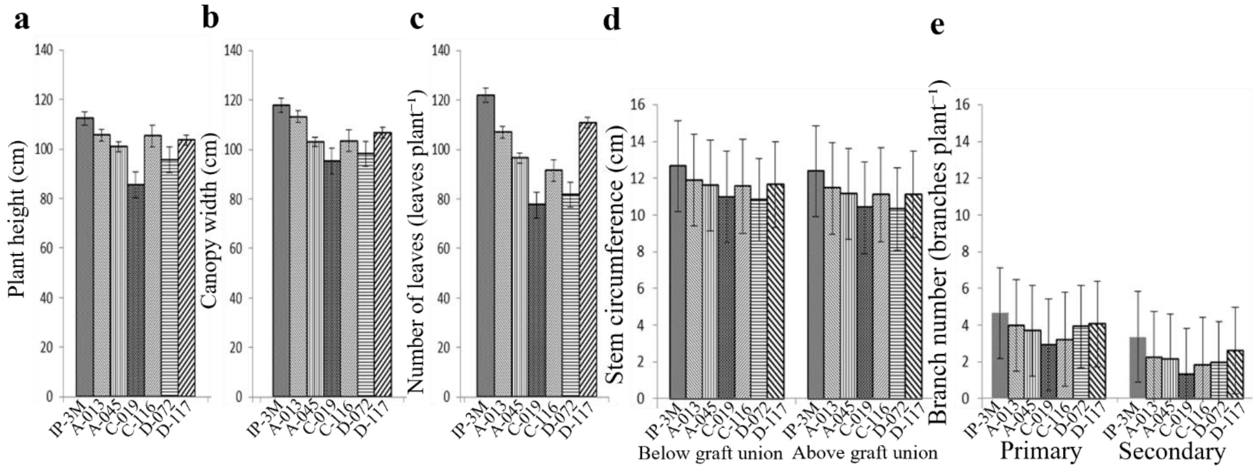

Figure 4.
Effect of rootstock accessions on vegetative growth traits(a) plant height, (b) canopy width, (c) leaf number, (d) stem circumference, and (e) branch number. Data are presented as mean ± standard error (SE).

The findings regarding the vegetative growth and production components of various accessions in a grafting system highlight significant differences among them, particularly in the context of rootstock selection.

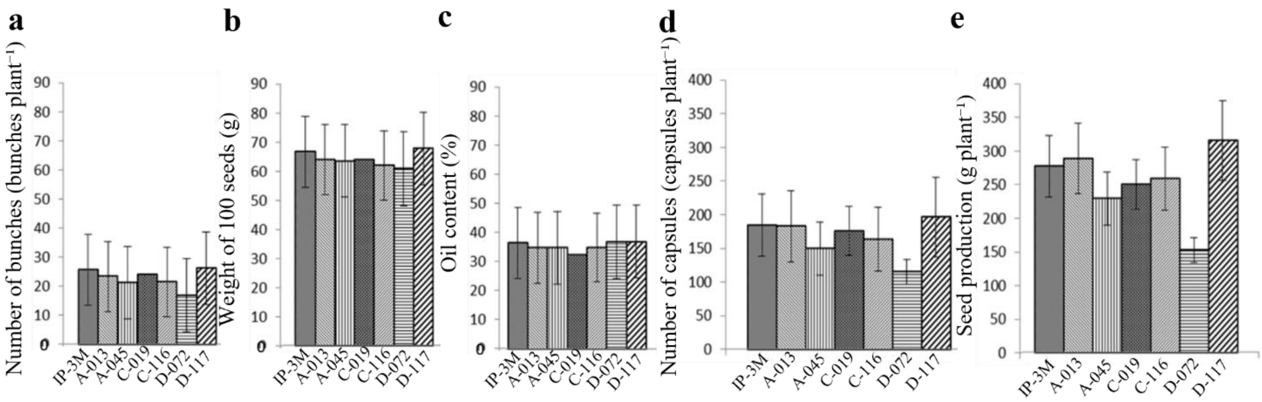

Figure 5.
Effect of rootstock accessions on production components and oil content: (a) bunch number, (b) weight of 100 seeds, (c) oil content, (d) capsule number, and (e) seed production. Data are presented as mean ± standard error (SE).

3.5. Effects of Graft Combination Scion/Rootstock on Proline Content

Observations indicate that the HS-49 scion accession exhibits the highest leaf proline content among a combination of seven rootstock accessions, followed by the PT-7 accession. Conversely, the IP-3A scion accession shows the lowest leaf proline content. The elevated proline levels in HS-49 serve as a response mechanism to drought conditions, highlighting its role in osmotic adjustment (Figure 6).

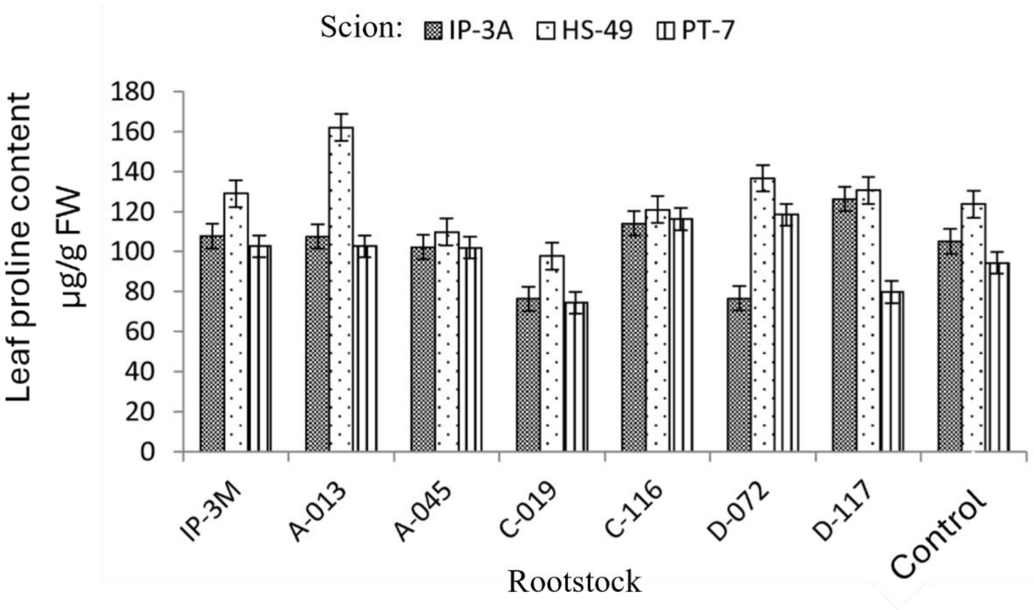

Figure 6.
Leaf proline content of different scion-rootstock graft combinations and the corresponding non-grafted scions (control). Data are presented as mean ± standard error (SE).

3.6. Effects of Scion and Rootstock on Proline Content

The A-13 rootstock displayed the highest concentration of leaf proline at 124.03 µg/g FW, whereas the C-116, IP-3M, and D-117 rootstocks exhibited lower, yet still significant, proline levels. The C-019 rootstock had the lowest leaf proline content at 82.87 µg/g FW. The HS-49 scion presented the highest leaf proline concentration at 126.28 µg/g FW, followed by the IP-3M scion, which showed a comparatively lower but still remarkable proline level. Conversely, the PT-7 scion had the lowest leaf proline concentration at 98.79 µg/g FW (Figure 7). This approach entails the preservation of water transport within the plant by augmenting the elasticity of cell walls, consequently alleviating the necessity for osmotic adjustment [24-27].

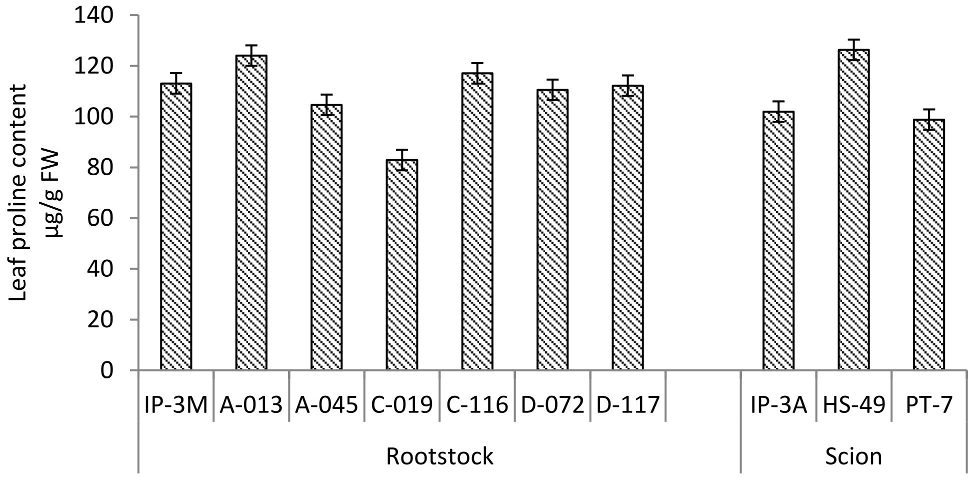

Figure 7.
Leaf proline content of different rootstocks and scions. Data are presented as mean ± standard error (SE).

3.7. Histological Analysis of Grafting

The scion and rootstock can unite by forming a callus and new vessel network as a bridge connecting the xylem and phloem vessel networks of the scion and rootstock. As an illustration of the results of transverse anatomical observations of the stem in the connection area can be seen in Figure 8. Histological observation allows for the examination of tissues at a microscopic level, which can reveal early signs of incompatibility, such as cellular damage or abnormal tissue formation, shortly after grafting [28].

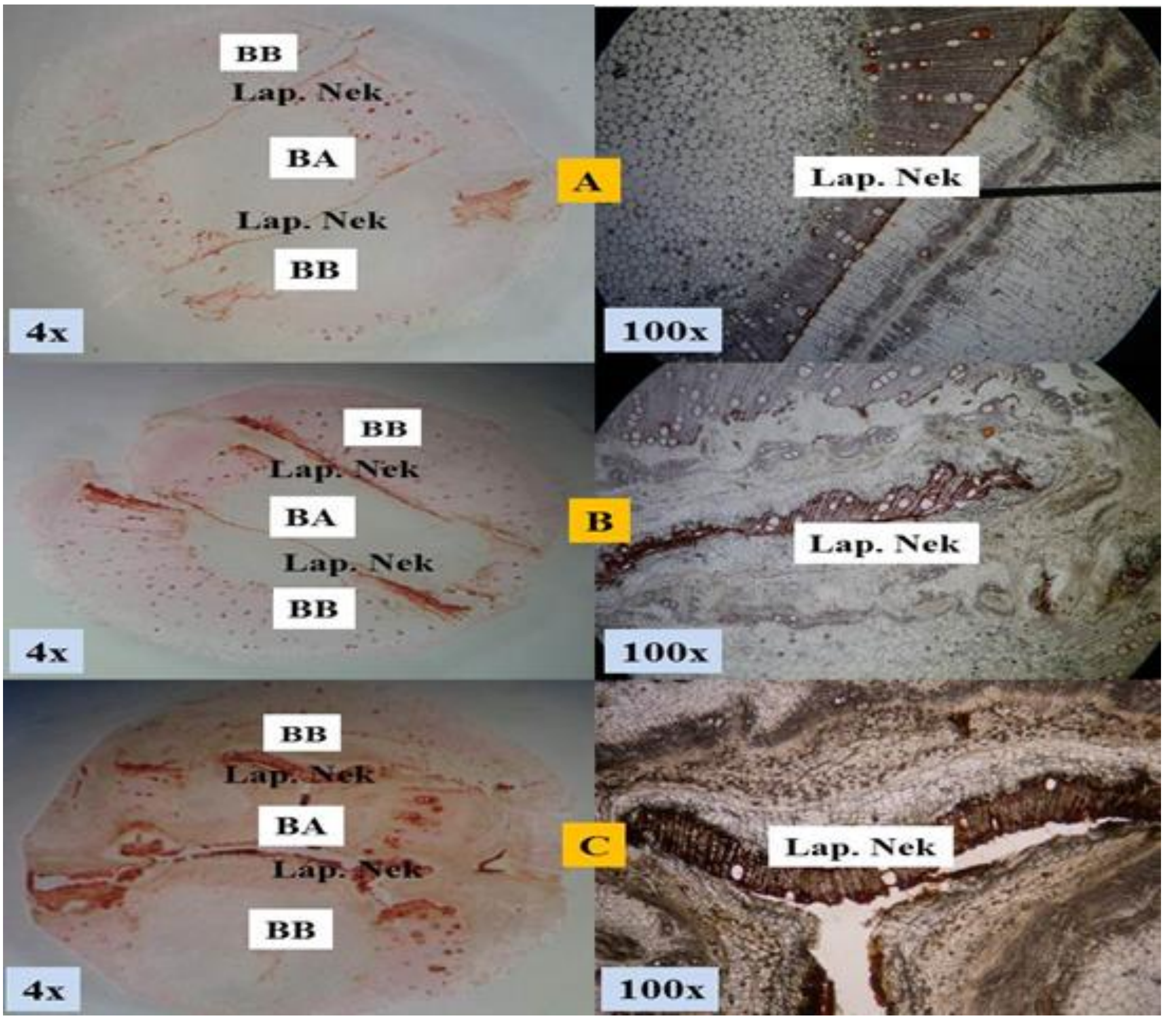

Figure 8.
Cross-section of the joint area between scion and rootstock. BA: scion, BB: rootstock, Lap.Nek: necrotic layer, A: PT-7/ Sulawesi 117 (compatible), B: HS-49/Jatim 045 (less compatible), C: IP-3A/ Sulawesi 027 (incompatible).

4. Discussion

Grafting involves attaching a piece of one plant (scion) to another plant (rootstock) to create a single, composite plant. Grafting can improve plant productivity by combining desirable traits from different plants. It enhances vigor, disease resistance, and abiotic stress tolerance without genetic modification [29]. These improvements suggest that grafting can lead to better growth and productivity in Jatropha plants, indicating the potential benefits of this technique in agricultural practices aimed at enhancing crop yields and quality [30]. The ability of two different plants, grafted together, to produce a successful union and to develop satisfactorily into one composite plant is termed graft compatibility. Grafted plants with high compatibility tend to grow and produce better than their original counterparts.

The findings presented in Figure 1 indicate that grafting scions onto rootstocks leads to superior performance in vegetative growth, component production, and oil content compared to non-grafted (autografted) plants. Studies have demonstrated that plants grafted onto such rootstocks exhibit better growth parameters and higher yields compared to those that are not grafted or are autografted [31-33]. This enhancement is attributed to the use of drought-tolerant These traits contribute to better nutrient and water absorption, which is crucial for plant health, resistant to environmental stress, would increase yield [31, 34, 35].

The use of IP-3M accessions as rootstocks grafted with the different scions (IP-3A, HS-49, and PT-7) obtained plant growth traits better than other rootstocks accessions as combined grafting plants, and control (non combined scion). A grafting combination of IP-3A accession as scion and IP-3M as rootstock gave plant growth traits better than other rootstock accessions as combined grafting plants, and IP-3A as non combined scion. Similar results were shown in a combination of grafting HS-49 and PT-7 accession as scion and IP-3M accession as rootstock give plant growth better than other rootstock accessions as combined grafting plants, and IP-3A as non combined scion.

Base on previouse study of rootstock showed that IP-3M had good performance viz. vigorous stem, deep root system dan wide root distribution, so better support to scion performance to reach better vegetative and generative growth, production and oil content. Root characteristics, especially root length, root length density, and the number of thick roots, are important for plants to develop their aboveground parts by exploiting the soil available water [36].

Thus, it can be noted that the type of interaction between the rootstock and the scion is very complex, though varying among genetically diverse plants. Theories advanced as possible explanations for the interaction between the rootstock and scion include: (a) anatomical factors; (b) nutritional and carbohydrate levels, (c) absorption and translocation of nutrients and water; (d) phytohormones and correlative effects and (e) other physiological factors. The morphological advantages provided by the rootstock, such as a more extensive and efficient root system, lead to improved nutrient uptake. This not only supports vegetative growth but also contributes to the overall yield and quality of the harvested produce [36].

The success of grafting is largely dependent on the compatibility between the rootstock and scion. Incompatible combinations often struggle to develop a well-functioning vascular system, which can lead to graft failure [28, 37]. The PT-7 accession's superior performance suggests a high degree of compatibility, which is crucial for successful grafting and enhanced plant productivity [17, 23, 38]. The effects of scion on plant growth traits, production, and oil content in grafted plants are multifaceted and influenced by the interaction between the scion and the rootstock. the scion's effects on plant growth traits, production, and oil content are highly dependent on the interaction with the rootstock. This interaction can enhance or hinder various aspects of plant growth and productivity, depending on the specific scion-rootstock combination [34, 39].

The formation of a successful graft union requires the integration of the scion and the rootstock. This integration is facilitated by the development of a meristematic area at the graft union, which allows for the regeneration of vascular tissues necessary for nutrient and water transport [17]. The process involves wound healing mechanisms, including callus formation and vascular tissue regeneration [29]. The IP-3M accession exhibited the best vegetative growth as a rootstock, followed by D-117, A-013, and C-116 accessions. This suggests that IP-3M is a superior choice for enhancing plant vigor in grafting systems. When evaluating production components and oil content, the D-117 accession emerged as the most productive, followed closely by IP-3M, A-013, and C-116. This indicates that while IP-3M is excellent for vegetative growth, D-117 may be more beneficial for maximizing oil yield. In summary, while IP-3M is recommended for its vigorous growth as a rootstock, D-117 may be preferred for its superior oil production characteristics.

The rootstock significantly affects the scion's growth traits. For example, a vigorous rootstock can enhance the growth of a less vigorous scion, but it can also reduce shoot growth in some cases, as seen in the study on sweet basil grafting where a more vigorous rootstock decreased the shoot dry mass and stem length of a less vigorous scion [39]. The rootstock can control the scion's growth through hormonal regulation. For instance, the rootstock can influence the production and translocation of plant hormones, which in turn affect the scion's growth and development [34]. Grafting can enhance the scion's tolerance to environmental stresses, this is because the rootstock can provide additional support and protection to the scion through improved nutrient uptake and stress mitigation mechanisms [39].

Osmotic adjustment (OA) is a crucial physiological mechanism that enables plants to adapt to water deficits by synthesizing and accumulating osmolytes, such as sugars, amino acids, and sugar alcohols [40, 41]. This process helps lower the osmotic potential in cells, allowing for water retention and maintenance of turgor pressure, which is vital for plant survival during periods of drought [42-44]. Studies have shown that plants exhibiting effective osmotic adjustment can maintain higher leaf turgor potentials at given leaf water potentials compared to non-adjusting counterparts. This capability allows them to sustain growth and productivity even under adverse conditions [45-47].

The effects of graft combinations on proline content in plants are not directly addressed in the provided sources. However, the interaction between the scion and rootstock can influence various physiological processes, including stress responses, nutrient uptake, and metabolic pathways, which can indirectly affect proline content. Proline, along with quaternary ammonium compounds such as glycinebetaine, choline, and prolinebetaine, are essential osmolytes that contribute to this process [26, 33, 48-51]. Proline is often accumulated in plants under stress conditions as a protective mechanism. Therefore, grafting combinations that improve stress tolerance might also influence proline levels indirectly by reducing the need for proline accumulation as a stress response [39, 52].

Proline accumulation is a critical adaptive response in plants to environmental stresses like drought, temperature fluctuations, and starvation [53]. By facilitating osmoregulation and maintaining cellular homeostasis, proline helps plants to buffer the immediate effects of water shortages and other stresses, thereby enhancing their overall resilience and productivity under adverse conditions [54]. Proline not only aids in osmoregulation but also protects cellular structures by stabilizing proteins and membranes during stress conditions. It acts as a scavenger of reactive oxygen species (ROS), thereby mitigating oxidative damage that can occur during periods of environmental stress [53].

The interaction between the scion and rootstock can influence metabolic pathways, including those involved in amino acid synthesis and degradation. Proline is synthesized through the glutamate pathway, and changes in this pathway due to grafting could affect proline content [52]. The ability of plants to accumulate proline in response to diverse stressors, such as drought, extreme temperatures, and nutritional deficiency, represents a critical adaptive strategy that enhances their overall resilience. By reducing water potentials and facilitating osmoregulation, proline assumes a fundamental role in enabling plants to endure adverse environmental conditions while sustaining growth and productivity.

Plants undergo acclimatization to drought stress through the implementation of various adaptive strategies, which encompass drought escape, drought avoidance, and drought tolerance [55-57]. The combination of drought escape and avoidance strategies enables plants to effectively manage water scarcity. By completing their reproductive cycle prior to the onset of drought conditions or by sustaining elevated tissue water potential via root modifications and diminished transpiration, plants can alleviate the detrimental impacts associated with drought stress [55, 56].

Drought avoidance involves maintaining relatively high tissue water potential despite reduced soil moisture. he accumulation of compatible solutes such as proline and sugars is instrumental in sustaining cellular turgor and osmotic equilibrium, thereby enabling plants to conserve water even in the face of stress (PMC) [57, 58]. Two fundamental defensive strategies are employed by plants to mitigate the effects of drought; the first involves the facilitation of water circulation within the plants, which is supported by a robust deep root system, while the second aims to minimize water loss through the regulation of transpiration and morphological changes.

The effects of scion and rootstock on proline content in grafted plants are significant, particularly in relation to stress tolerance mechanisms. Proline is an important amino acid that accumulates in plants under stress conditions, serving as an osmoprotectant and stabilizing cellular structures [27]. The interaction between scion and rootstock can lead to reciprocal effects on metabolite profiles, including proline. Research has shown that rootstock can directly affect the metabolic composition of the scion, which includes primary and secondary metabolites related to stress responses. This indicates that specific combinations may enhance or diminish proline levels depending on their compatibility and physiological characteristics [52].

The analysis of leaf proline content reveals significant differences among various rootstocks and scions in response to drought stress [31, 33, 41, 59]. Research demonstrates that a variety of solutes, encompassing inorganic cations, organic acids, carbohydrates, and amino acids such as proline, significantly contribute to osmotic adjustment (OA). Proline is particularly significant due to its dual role as both an osmoticum and an osmoprotectant, facilitating the stabilization of proteins and membranes under conditions of physiological stress [42, 56, 60]. However, the effectiveness of OA can vary among plant species; for instance, some species may rely more on increased cell wall elasticity rather than osmotic adjustment to cope with drought [56, 57]. Studies have shown that while proline can help mitigate damage from reactive oxygen species (ROS) and stabilize cellular structures, its presence does not always correlate with improved drought tolerance across all species [56, 60, 61].

Graft compatibility is influenced by complex physiological, biochemical, and anatomical interactions. Successful grafting relies on several factors including cellular compatibility systems, formation of plasmodesmata, and vascular tissue connections. The literature indicates that these factors play a crucial role in determining the success of graft unions between scions and rootstocks [18, 62, 63].

During the process of combining grafting and forming a vessel system, the suitability of the grafting is not known with certainty. Through macroscopic observation, waiting for a long time to determine the joining process can cause loss of time, cost and effort. Histological observation allows for the examination of tissues at a microscopic level, which can reveal early signs of incompatibility, and provides quick information on grafting compatibility [19, 37].

The results showed differences in gaps and necrotic spots that varied in the combination of connections, where the combination of compatible connections showed narrower gaps, and necrotic spots that showed less tissue damage. Histological observations of the combination of PT-7 joints with Sulawesi 117 rootstocks showed fewer necrotic spots, as well as the ability to regenerate and reunite the vessel network in the scar tissue at the joint junction faster than the incompatible joint combination. By examining transversal sections of the graft union, histological studies can identify specific histopathological changes associated with different grafting methods and infection types. This helps in understanding the mechanisms of graft union healing and potential issues like rot symptoms.

Callus formation can also repair necrotic layers by regenerating damaged cells, especially at the junction. The gaps still visible in the observations made indicate that the process of callus formation and differentiation into cambium and vascular tissue is still ongoing. The formation of new cambium networks/vessels allows the process of nutrient and water translocation from the rootstock to the scion, as well as the translocation of assimilates from the scion to the rootstock to take place normally again to support the growth and production of Jatropha japonica connection plants. Histological studies can elucidate the mechanisms of tissue reunion at the graft interface, including changes in cell division, the formation of plasmodesmata, and vascular connection. These processes are critical for the successful integration of the scion and rootstock [19, 28, 37].

Grafting is the actual process of pressing the cambium layer of the scion against the cambium layer of the stock, so the two layers will grow together. The alignment of the two cambium layers is critical to the success of grafting. Complete alignment of the cambium layers (CA) between scion and rootstock leads to higher success rates in grafting, as demonstrated in grapevines [64]. Proper alignment facilitates the formation of a callus bridge and vascular connections, essential for nutrient and water transport [17, 65] and promoting better compatibility and growth [28].

5. Conclusion

Among the accessions evaluated, PT-7 emerged as the most suitable source for scion in grafting systems, followed by HS-49 and IP-3A. Regarding rootstock selection, the IP-3M accession demonstrated the most favorable vegetative development, with D-117, A-013, and C-116 also showing considerable potential.

The integration of PT-7 as a scion with a suitable rootstock is projected to augment both morphological growth attributes and comprehensive crop yield in suboptimal abiotic conditions. This approach aligns well with the characteristics of lands suitable for Jatropha cultivation, facilitating its development as a viable crop in areas prone to drought conditions. Histological observation enhances the efficiency of grafting compatibility testing by offering detailed, early insights into the compatibility of the graft union, which can lead to more informed decisions and potentially better grafting outcomes.

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