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Article

Effects of Establishment on Growth, Yield, and Silage Qualities of Amaranth in Typhoon-Prone Southern Kyushu, Japan

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Abstract: Amaranth (*Amaranthus hypochondriacus* L.) is a potential forage crop with a high yield and crude protein (CP) content; however, establishment methods need to improve for the crop to be less sensitive to typhoons. Optimal establishment, cultivation, and utilization in amaranth were examined in a variety of seasons and methods of establishment in 2021–2023. Four methods were examined: (1) direct seeding in rows, (2) direct seeding in spots, (3) soil seed balls, and (4) transplant pretreatment methods under a randomized blocked design (n = 3). Sowings every month from April to August were applied only in 2021, while establishments in April, May, and August with both pretreatment methods were applied in 2022 and 2023. The establishment in August successfully escaped damage from typhoons. The direct seeding of either rows or spots showed marginal success in establishment compared to stable establishment in pretreatment methods. In 2022 and 2023, the highest yield and CP content were achieved in soil seed balls plots in April and in both pretreated plots sown in August, respectively. The quality of silage fermentation showed a high pH, ranging from 4.52 to 6.39, due to the high CP content in 7.59–18.36% dry matter (DM). Sowing in April or August established with soil seed balls can avoid typhoon damage to have stable forage yields and can be processed with a favorable quality of amaranth silage in the region.

Keywords: amaranth; forage quality; pretreatment methods; fermentation quality; sowing season



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1. Introduction

With the progress of global climate change, a risk of failure in soiling crops such as maize (*Zea mays* L.) should increase due to the impact of typhoon and heavy rainfall, and needs of growing alternative crops, as well as improving quality forage supply, could increase due to the high resilience to climate change [1]. Amaranth (*Amaranthus hypochondriacus* L.) is an annual and potential C₄ food crop, as well as a forage crop [2], that elongates to 2.5 m height, has a high yield and a high content of crude protein (CP) due to highly differentiated inflorescences, and should alleviate the general problem of CP deficiency in herbage production [3]. The substitution of maize silage for amaranth silage due to lower water requirements is now a hot topic for the feeding of quality herbage in the water deficit area of tropical and subtropical regions [4–7]. In addition, the cultivation of amaranth has been attempted in both a hot–humid climate [8] and a temperate climate in Lithuania [9], with amaranth processed for medium-quality silage. The superior potential of the high yield and CP content species for herbage use in amaranth was confirmed during spring sowing in southern Kyushu, located in the humid and warm region, by comparing amaranth with four species of conventional small-grain forage crops: foxtail millet (*Setaria italica*), Japanese barnyard millet (*Echinochloa esculenta*), proso millet (*Panicum miliaceum*), and forage sorghum (*Sorghum bicolor*) in the previous study [unpublished]. However, since the risk of lodging damage in amaranth from typhoons was also detected in the previous

study, it is necessary to avoid damage from typhoons [10] to establish stable amaranth production in southern Kyushu, Japan.

Typhoon damage causes stagnant seedling growth in summer crops, resulting in a decrease in forage yield and quality, which leads to an increase in production cost and unstable herbage supply due to an increased difficulty in mediating harvest and processing processes [11,12]. The southern Kyushu region is in a typhoon-prone area, with typhoons approaching and landing very frequently in the period from July to September, which is the main limiting factor for the cultivation of summer-sown crops such as maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* Moench) [13]. Therefore, one of the techniques for avoidance from typhoon damage is to harvest and process the first forage crop before the hot typhoon season from August to September [14]. In another approach, Li et al. [15] examined that the cultivation of blast-disease-resistant Italian ryegrass (*Lolium multiflorum* Lam.) can be sown in mid-September, which can replace summer-seeded maize production when the typhoon lodges the maize seedlings, resulting in the replacement of maize cultivation.

Li et al. [16] also examined that the sowing seasons of pearl millet (*Pennisetum typhoides*) shifted from mid-May to late June and late July to early September, clarifying that grass species achieved a dry matter (DM) yield of around 12 Mg ha⁻¹ at 8–11 weeks after sowing during the four sowing seasons. Therefore, one of the other cultivation techniques is to shift the amaranth sowing season from spring to the late summer season to avoid damage from typhoons. However, the shift in the sowing seasons has some problems to be solved; the first is the phenological development in amaranth, since the genotype exhibits prolonged vegetative growth under long-day conditions [17]. The second is the processing method after harvest, since the shift in the sowing seasons to mid-summer results in the harvest of amaranth in late autumn, when the air temperature declines and the processing of hay is so much more difficult at lower temperatures than in the summer season that ensiling wilted amaranth should be the best processing and storage alternative [18].

A high moisture content makes it difficult to achieve a good silage quality in amaranth [19,20], such as an increase in lactic acid content, concomitant with a decrease in acetic acid content and ammonia nitrogen/total nitrogen ratio [20]. Ma et al. [21] revealed that, when looking at harvest in the various growth stages of amaranth, the full maturity stage should be the best for harvesting and processing both hay and silage production due to the better forage qualities, with an increasing starch content in inflorescence. Therefore, it is also necessary to examine how the maturity of the inflorescence progresses in the amaranth when it is seeded in mid to late August.

Pre-sowing treatments are often applied to improve germination and established rates of small seeds of native plant species such as physical sandpaper, acid and cold scarification treatments, and seed presoaking treatments, which showed accelerated germination for 75% of species examined [22]. One of the other presoaking methods is seed balls, which need a lower cost compared with transplants of seedlings and can protect sowing seeds from attacks by birds, ants, and unfavorable stresses, even without land preparation [23].

Due to the limited history of amaranth cultivation for forage use in southern Kyushu, the southernmost main islands of Japan, the objectives of the current study were to clarify the suitable establishment, cultivation, and utilization methods by comparing the quantity of amaranth in terms of establishment rate, shoot density, plant height, lodging, and fractionated plant dry weight, quality of amaranth, such as in vitro dry matter digestibility (IVDMD) and CP content, silage fermentation quality, such as the pH, and contents of lactic acid, volatile fatty acids, ammonia nitrogen, and total nitrogen in a variety of sowing and establishment seasons from April to August and using establishment methods that include direct seedings, transplants, and soil seed balls in the region.

2. Materials and Methods

2.1. Study Area

Field experiments from 2021 to 2023 were conducted at the Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan (31°49'40" N, 131°24'46" E, 28 m a.s.l.).

The meteorological data, such as temperature, rainfall, and typhoons, were obtained from the Japan Meteorological Agency [24]. In 2021, the experimental period was from mid-April to early December, when the mean temperature was 21.9 °C and the cumulative rainfall was 2611 mm. Typhoon No. 9 in 2021 approached in early and mid-August. The numbering of typhoons was announced by the World Meteorological Organization. The rainfall distribution was abundant in mid-May, mid-August, and mid-September, with limited rainfall from late August to early September. The high autumn temperature occurred from late September to mid-October, when the mean temperatures of 10 days were maintained above 22 °C (Figure 1A).

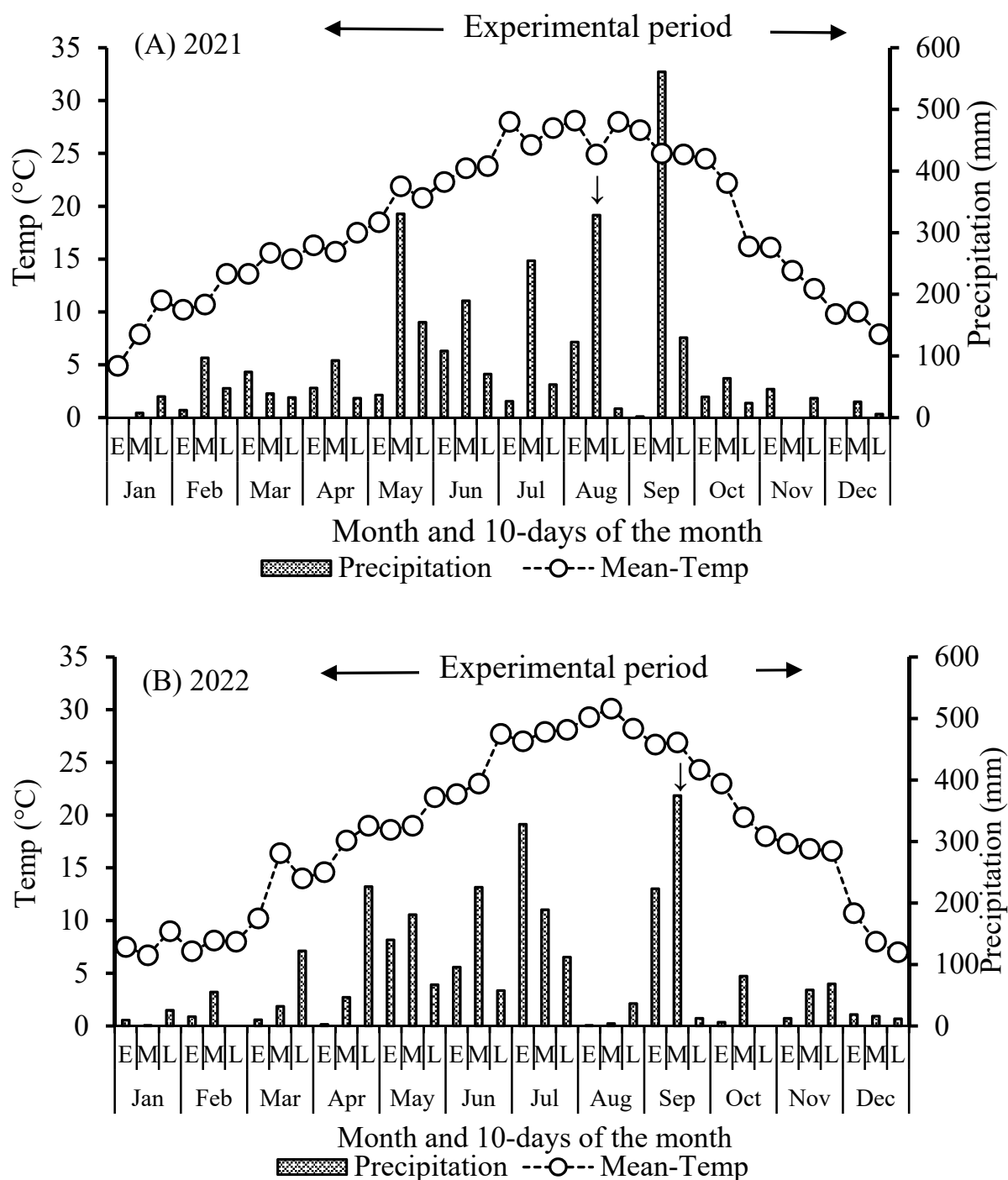


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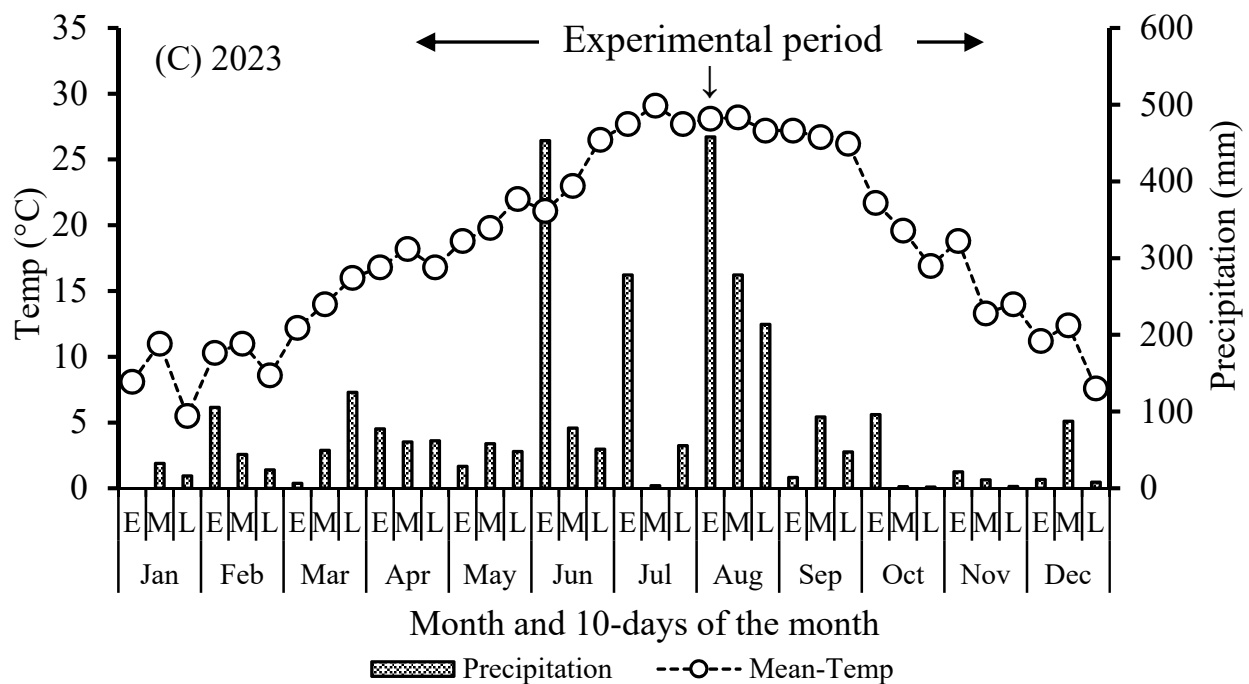


Figure 1. Changes in daily mean temperature and cumulative precipitation in the ten days of the month in the season of 2021 (A), 2022 (B), and 2023 (C). Ten days of the month: early (E), mid (M), and late (L). The arrow ($\downarrow$) shows the time of typhoon attack (No. 9 in 2021, No. 14 in 2022 and No. 6 in 2023).

In 2022, the experimental period was from early April to early November, when the mean temperature throughout the periods was 23.2 °C and the cumulative rainfall was 2422 mm. Precipitation was relatively abundant and uniform from late April to late July compared to the other two years. Except for the peak of rainfall in mid-September due to the landing of Typhoon No. 14 in 2022, the amount of precipitation was lower in the period from late September to early November compared to the first half of the season, and the temperature of the autumn decreased sharply after mid-September (Figure 1B).

In 2023, the experimental period was from mid-April to mid-November, when the mean temperature between the periods was 22.9 °C and the cumulative rainfall was 2413 mm. In the first half of the season, except for abundant rainfall in early June and early July, precipitation was uniform, but lower than in the other two years. Typhoon No. 6 in 2023 approached in early August, when the maximum rainfall occurred, followed by continuous rainfall until early October, which might be the detrimental factor for the May planting. The irregularly high temperature was stagnant from early August to late September, which could stimulate the growth of amaranth (Figure 1C).

2.2. Experimental Design and Forage Preparation

The establishment, growth, and yield in amaranth were examined in the volcanic ash soils without irrigation from 2021 to 2023. Three to five establishment seasons were examined from early April to early November in the periods of 2021 to 2023. One week before planting amaranth, fermented manure at 30 t ha⁻¹ and dolomite at 1.5 t ha⁻¹ were applied to the field plots as a base fertilizer, followed by plowing with a cultivator at 20 cm depth. The plots were assigned to a randomized plot design with three replications and the size of each plot was 5 m² (2 m × 2.5 m).

The seasons, sowing and establishment methods, and harvest stages in the amaranth are listed in Table 1. Direct seeding in rows means seeding in rows by hands, while direct seeding in the spots means seeding with the constant plant spacing of 40 cm × 40 cm in 2021; however, the spacing was unified to be 30 cm × 30 cm (11.11 plants m⁻²), which was

the recommend density by the seeding company (Marche Aozora Co., Ltd., Nagoya, Japan). As for pretreated methods in amaranth, transplant plots were seeded in 5 cm paper pots 4–6 weeks before the establishment and the seedlings were transplanted into the plot at the establishment. Treatment as soil seed balls involved sowing 4–5 seeds in the 2 to 3 g peat ball surface layer indoors for 1 day followed by transplanting the balls into the field, resulting in more vigorous emergence [23] since the peat clay maintained the moisture for a good establishment of seedlings.

Table 1. Seasons, establishment methods, and harvest stages in amaranth in 2021–2023.

Year	Date of Establishment	Method of Establishment	Abbreviation of Establishment	Plant Spacing	Sowing Date	Harvest Stage of Amaranth
2021	14 April	Direct seeding in row Direct seeding in spot *	April-Row April-Spot	40 cm † 40 cm × 40 cm	14 April 14 April	Late flowering
	11 May	Direct seeding in row Direct seeding in spot	May-Row May-Spot	40 cm † 40 cm × 40 cm	11 May 11 May	
	29 June	Transplants Direct seeding in spot Soil seed balls	June-TransP June-Spot June-SoilB	40 cm × 40 cm 40 cm × 40 cm 40 cm × 40 cm	11 May 29 June 26 June	
	21 July	Direct seeding in spot Soil seed balls	July-Spot July-SoilB	40 cm × 40 cm 40 cm × 40 cm	21 July 20 July	
	30 August	Soil seed balls	August-SoilB	40 cm × 40 cm	29 August	
2022	9 April	Transplants Soil seed balls	April-TransP April-SoilB	30 cm × 30 cm 30 cm × 30 cm	14 March 8 April	Full maturity
	22 May 23 May	Transplants Soil seed balls	May-TransP May-SoilB	30 cm × 30 cm 30 cm × 30 cm	25 April 22 May	
	11 August	Transplants Soil seed balls	August-TransP August-SoilB	30 cm × 30 cm 30 cm × 30 cm	18 July 10 August	
2023	11 April	Transplants Soil seed balls	April-TransP April-SoilB	30 cm × 30 cm 30 cm × 30 cm	15 March 9 April	Full maturity
	23 May	Transplants Soil seed balls	May-TransP May-SoilB	30 cm × 30 cm 30 cm × 30 cm	20 April 22 May	
	19 August	Transplants Soil seed balls	August-TransP August-SoilB	30 cm × 30 cm 30 cm × 30 cm	17 July 18 August	

Note: fertilizer rate was fixed at 10 g m⁻² season⁻¹ for each of N, P₂O₅, and K₂O. † Spacing of the rows in direct seeding. * Spot means seedling with constant plant spacing.

The weeding was carried out manually. The topdressing was applied with N-P₂O₅-K₂O chemical fertilizer (14-14-14) three times before the flowering stage to give a total of 100 kg ha⁻¹ of each element for all treatments.

The height and density of the plants were recorded for 2 plants in each of the replicate plots (total of 6 plants per treatment) at intervals of 1 or 2 weeks. The weights of fresh matter (FM) of each plant fraction, including leaves, stems, and inflorescences, were measured cut 5 cm above the soil surface using 2 plants from each of the replicate plots (total of 6 plants per treatment) at the time of harvest (flowering stage or maturity). The DM weights of each plant fraction were determined by oven drying at 70 °C for more than 72 h, and the DM yield was calculated by the total plant DM weight, which was the sum of the DM weight of each fraction multiplied by the density of the plant. The crop growth rate (CGR) was calculated by sampling to detect DM weights of the whole plant in 2 plants in each of the replicate plots (total of 6 plants per treatment) around three weeks before harvest and at harvest.

2.3. Silage Preparation

For July and August established plots in 2021 and only for August established plots in 2022, amaranth was pre-dried under the sunshine for one–two days and then ensiled in a

30 L plastic silo and kept indoors at an ambient temperature for more than 3 months. Silage sampling was performed at 200–300 g of fresh weight (FW) when the silo was opened.

2.4. Forage Quality Analysis for Plant Samples and Silage

The IVDMD of each plant fraction was determined using a pepsin–cellulase assay [25] using a filter bag method and an in vitro incubator (ANKOM Technology Co., Ltd., Macedon, NY, USA). The nitrogen content of each plant fraction at harvest was measured by the Kjeldahl method [26] and the CP content was calculated by the nitrogen content multiplied by 6.25 [27].

For the quality attributes of the silage, 50 g of each silage sample was dried to be constant at 70 °C to determine the DM content and the CP content of the silage. A total of 40 g of each silage sample was cut into 1 cm pieces and then soaked in 360 mL of distilled water for 12 h, and the mixture was filtered with Filter Paper No. 5A to obtain the filtrate. The pH of the filtrate was measured with a pH meter (AS800, AS ONE Corporation, Osaka, Japan), and the remaining samples were used to determine the contents of lactic acid and volatile fatty acids (VFAs) such as acetic acid, propionic acid, butyric acid, and valeric acid. Lactic acid was determined by the colorimetric method using a spectrophotometer (UVmini-1240, SHIMADZU Corporation, Kyoto, Japan) based on [28]. Volatile basic nitrogen (VBN) was determined by steam distillation and titration [29]. The VFA content was determined by gas chromatography (column filler: FAL-M, SHIMADZU Corporation, Kyoto, Japan).

2.5. Statistical Analysis

Statistical analyses were performed to compare growth attributes, DM yield, IVDMD, and CP content for all establishment methods from 2021 to 2023. Differences in mean values between methods were compared using a one-way analysis of variance, at 5% probability, by multiple comparisons with Duncan's test using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Establishment Rate

The rate of establishment of amaranth for four establishment methods in 2021 is shown in Figure 2A. A higher establishment rate was observed in the pretreatment transplants and soil seed balls plots than in the direct seeding of spot and row plots, while, among the two pretreatment plots, the rate showed greater variation. Throughout the months of establishment in 2022 and 2023, the rate of the pretreatment plots tended to increase from April to August, which could be due to the increase in temperature shown in Figure 2B. However, the variation in the rate of establishment reduced among the pretreatment plots in both 2022 and 2023 compared with that in the four established methods in 2021.

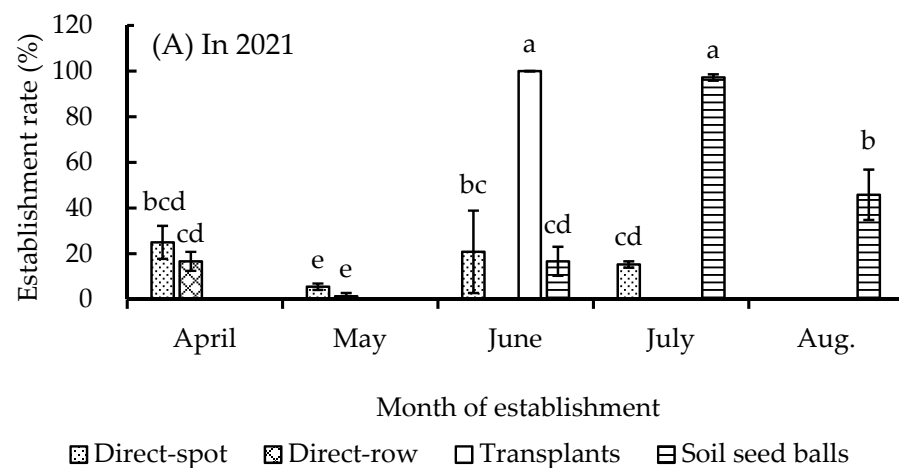


Figure 2. Cont.

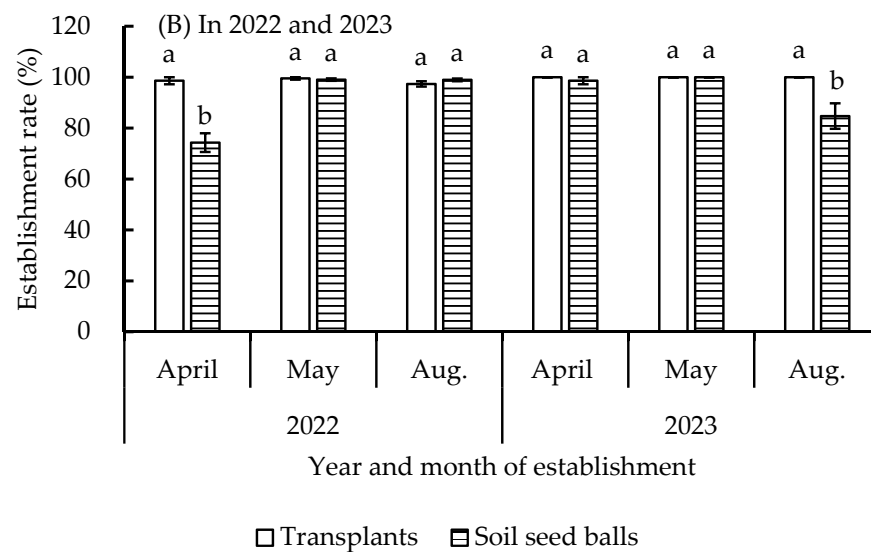


Figure 2. The rate of establishment of amaranth in a variety of months and establishment methods in (A) 2021 and in (B) 2022–2023 (mean $\pm$ standard error, $n = 3$). Distinct lowercase letters signify significant differences between establishment methods at $p < 0.05$.

3.2. Changes in Shoot Density

The plant density was lower at 6.67 m^{-2} in 2021 (Figure 3A) than at 11.11 m^{-2} in both 2022 (Figure 3B) and 2023 (Figure 3C). Therefore, the density of the amaranth shoots was higher in 2022 and 2023 than in 2021. When the peak density reached 2–3 weeks after establishment, the density decreased dramatically in the August planting plots in 2022 due to their sensitivity to typhoon damage and strong apical dominance, resulting in the breakage of the shoots, causing the decline in density (Figure 3B). In 2021, the density continued to decrease when direct seeding was applied in the spot or pretreatment of soil seed balls in June, when the amaranth harvest failed (Figure 3A). Typhoon damage occurred in the stem elongation stages about 30–60 days after establishment, and was the most severe throughout the growth stage due to the fragile stems and increased plant height compared to the earlier seedling stage, leading to stem breakage and failure in harvest. In 2023, the maximum density of the shoots was maintained throughout the periods over months and the methods examined (Figure 3C).

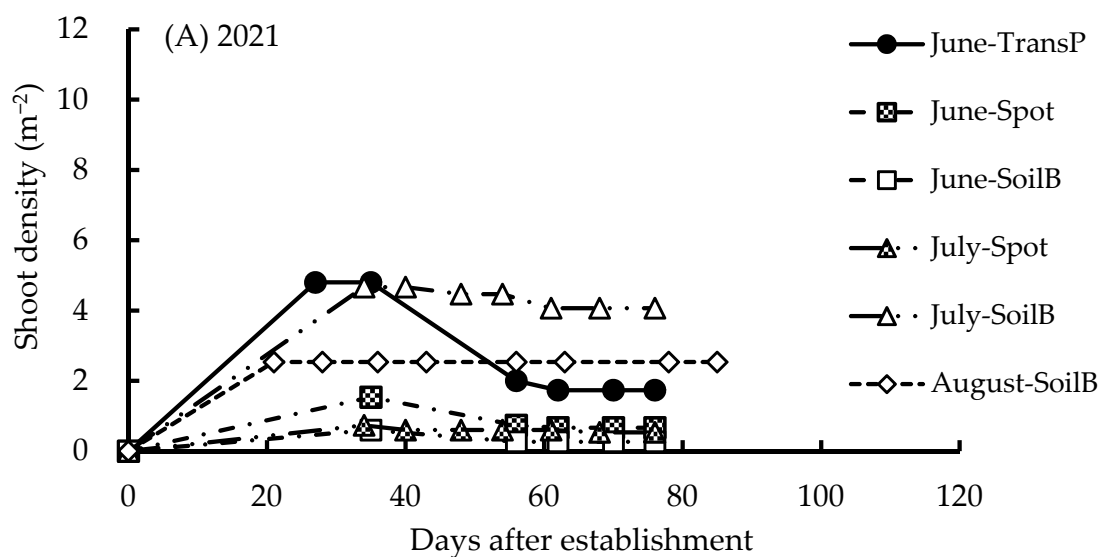


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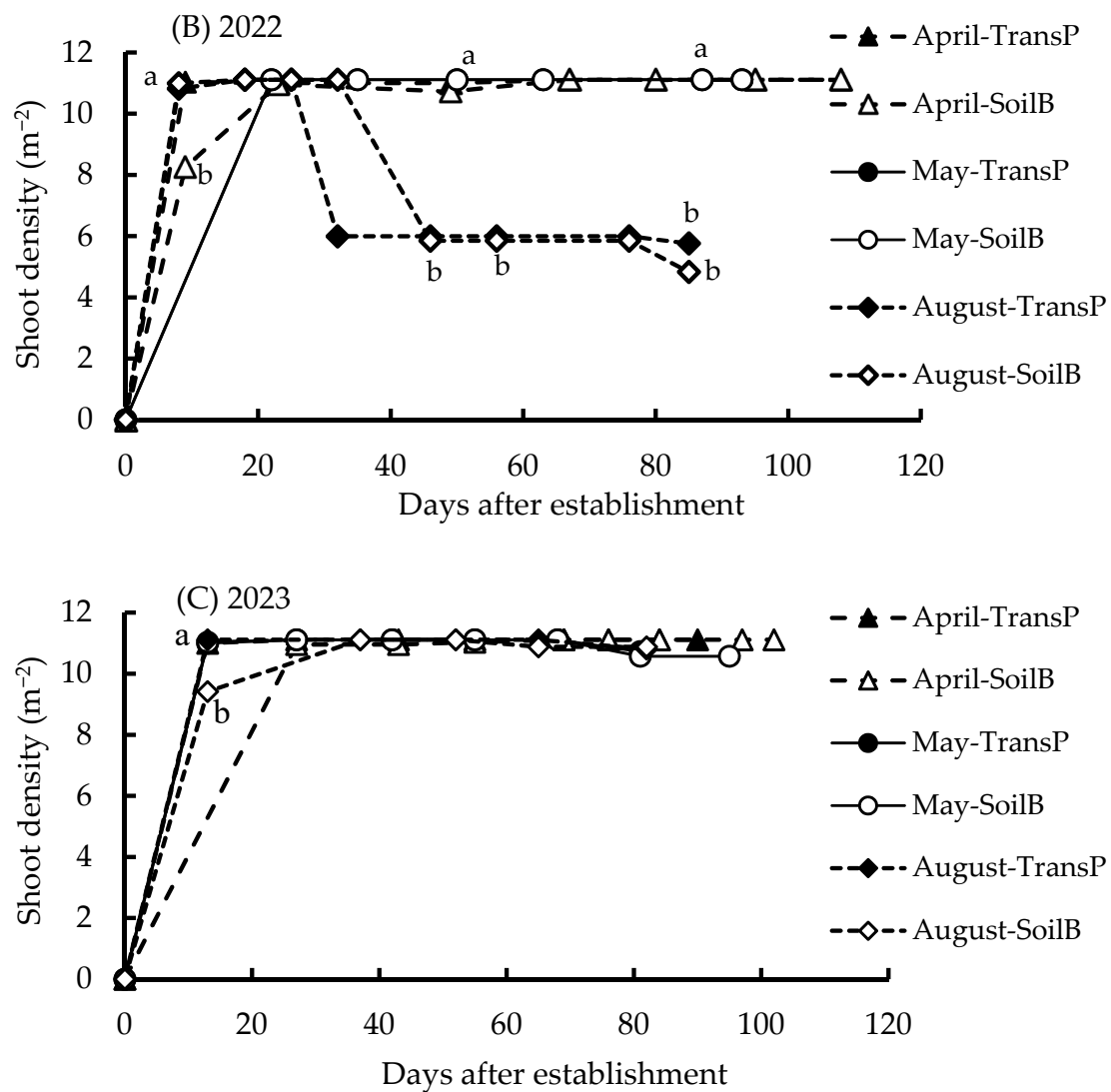


Figure 3. Changes in shoot density with days after establishment of amaranth under different establishment methods in 2021 (A), 2022 (B), and 2023 (C). Symbols followed by the same letter are not significantly different between methods at the 5% level of probability by the Duncan's multiple range test for the 2022 and 2023 experiments.

3.3. Changes in Plant Height

The height of the plants in the amaranth tended to be lower, at around 150 cm, in 2021 than in 2022 and 2023, at around 200 cm, without damage from a typhoon or heavy rain. In 2021, the establishment with direct seeding in the spot or pretreated soil seed balls in June reduced the height of the plant due to heavy rainfall in the establishment (Figure 4A). In the case of establishment in July, the height of the plant in soil seed balls increased at the same rate as in transplants in June and in soil seed balls in August, followed by direct seeding in the spot (Figure 4A).

In 2022, the increase in plant height showed a large variation with seasons and methods. Establishment in May showed a higher plant height than the other two months. Compared to established methods, transplants tended to be higher than plots in soil seed balls, while the difference tended to be smaller at harvest, except for establishment in August (Figure 4B).

In 2023, the variation in plant height between seasons and methods was reduced from that in 2022, and the increasing rate in plant height tended to be lower in the plots

established in April than in the other two months, while the plant height at harvest was almost similar, at a range of 180–220 cm (Figure 4C).

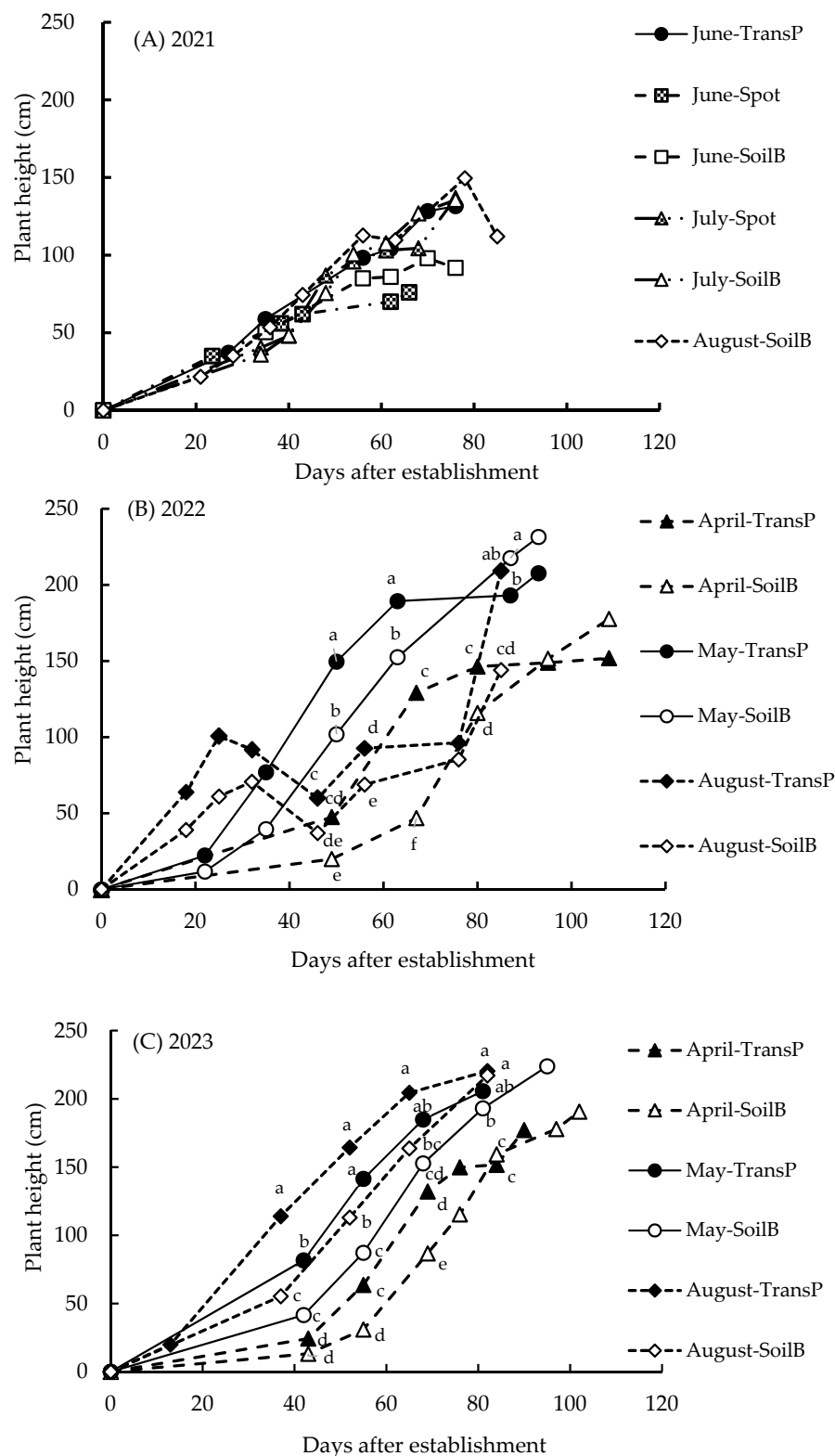


Figure 4. Changes in plant height with days after establishment of amaranth under different establishment methods in 2021 (A), 2022 (B), and 2023 (C). Symbols followed by the same letter are not significantly different between methods at the 5% level of probability by the Duncan's multiple range test for the 2022 and 2023 experiments.

3.4. DM Yields and CGR in 2021–2023

Based on the phenological changes in shoot density and plant height, the DM yields in whole plants, as well as in each plant fraction at harvest, are listed in Table 2. The DM yields were variable between the established years, where they were lower in 2021, except for soil seed ball plots sown in July, than in the other two years. The DM yields ranged from 1460 to 2040 and 1300 to 1890 g m⁻² in 2022 and 2023, respectively, except for the plots established in August 2022 due to severe lodging from typhoon damage (Table 2B). The soil seed ball plots showed higher or almost similar DM yields as in transplants in 2022 and 2023 (Tables 2B and 2C, respectively). The partitioning of DM among plant fractions showed variation between the months of establishment, when the highest accumulation of DM in the inflorescences was observed in the established plots in April, followed by those in May, and the lowest in the establishment of August. The DM partitioning to the inflorescence was reflected with the maturity stage, and was almost at the full maturity stage in the April plots compared to the still full-bloom stages in the August plots in 2022 (Table 2B). The degree of damage caused by typhoons in lodging was very severe in the plots established in June 2021 and August 2022, when harvest almost failed or suffered severely in the former and latter seasons, respectively.

The changes in CGRs on days after establishment are shown in Figure 5, where the significant positive correlation ($p < 0.01$) is obtained every year. These correlations suggest that the CGR in amaranth increased dramatically around 40 days after establishment to reach above 50 g m⁻² day⁻¹ in 2022 and 2023 (Figure 5B,C, respectively). The highest CGR revealed was in the soil seed balls sown in April in 2022 and 2023.

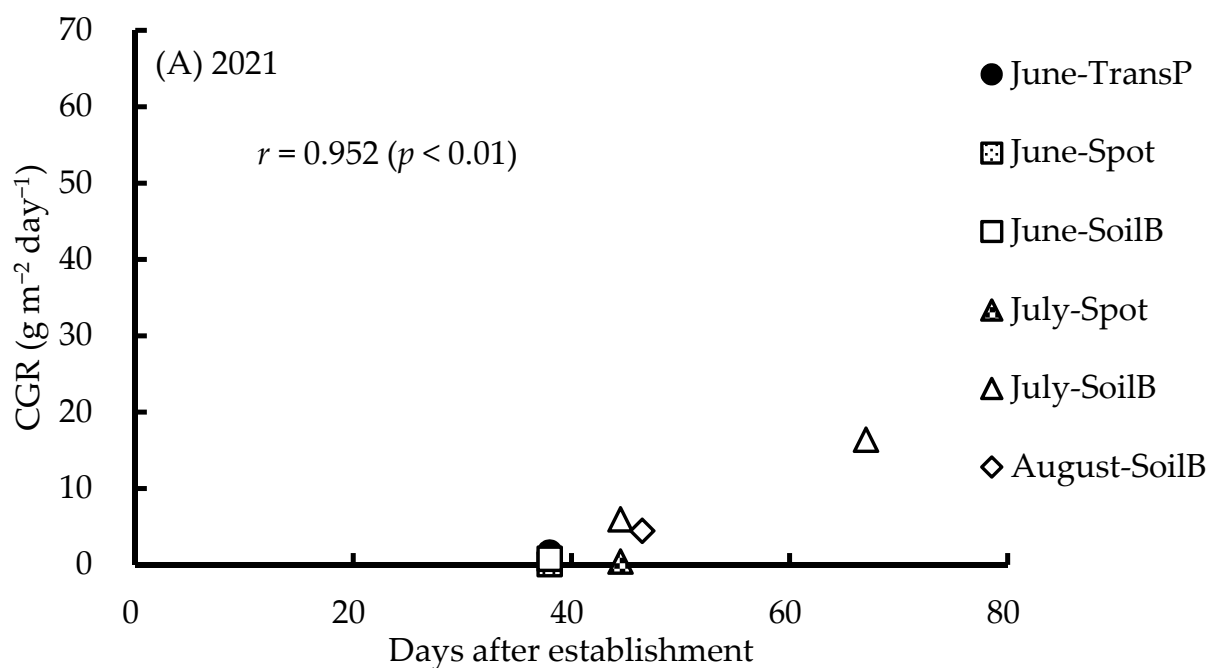


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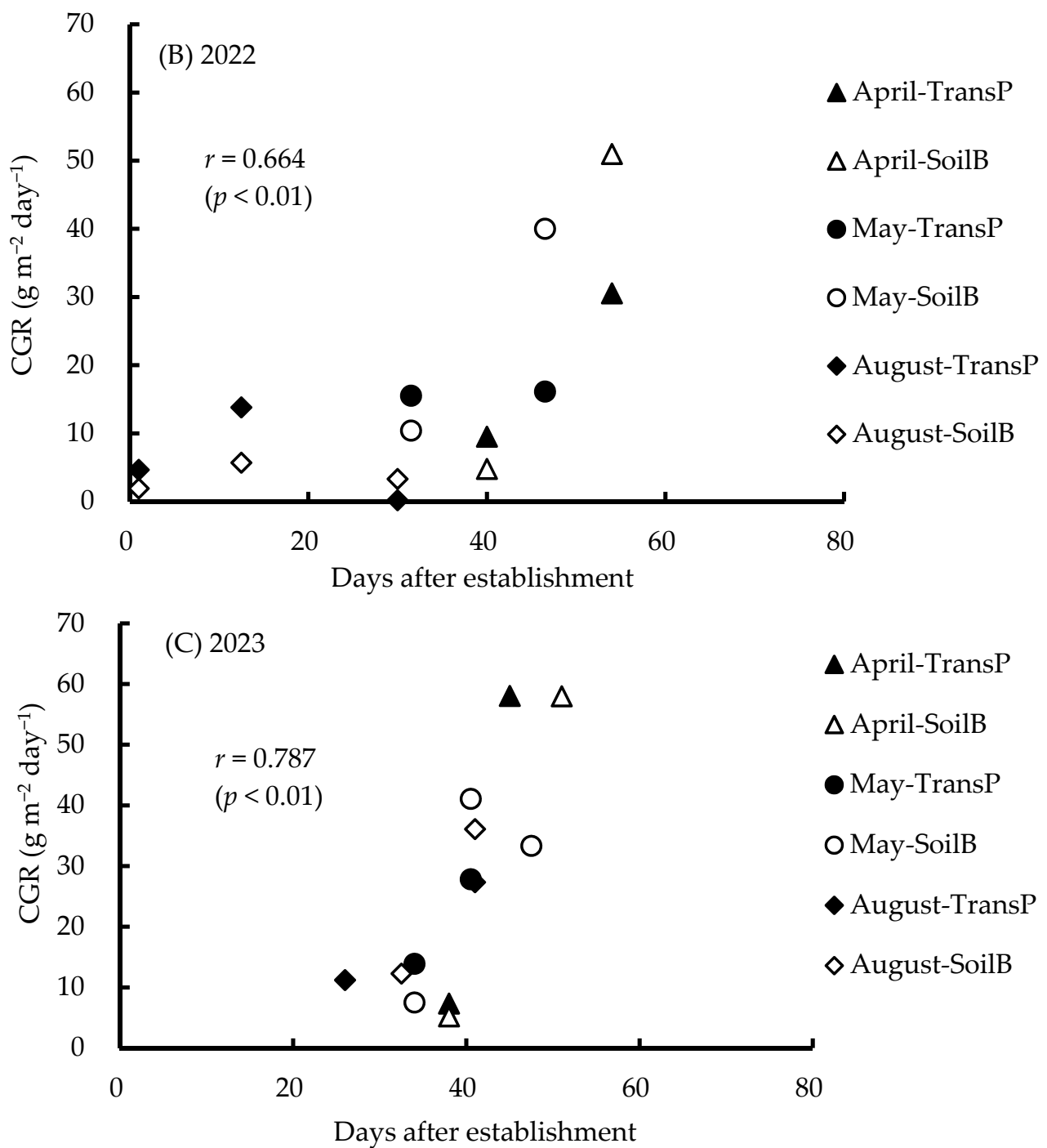


Figure 5. Relationship between days after establishment and crop growth rate (CGR) in the first harvest plants in 2021 (A), 2022 (B), and 2023 (C).

Table 2. Harvest date, maturity, height and density of the plant, average fractionated dry matter weight at harvest, and damage to the plant by typhoons in a variety of establishment methods in 2021 (A), 2022 (B), and 2023 (C).

(A) 2021										
Establishment Method	Harvest Date	Maturity at Harvest	Plant Height (cm)	Plant Density (m ⁻²)	Fresh Yield (g m ⁻²)	Dry Matter Yield (g m ⁻²)				Lodging by Typhoon (%)
						Leaves	Stems	Inflo-Rescences	Whole Plants	
June-TransP	13 September	Early seed mature	131.6	1.8	942.9	36.0	63.9	25.1	125.0	58.3
June-Spot		Full-bloom	66.0	0.7	1.7	—	—	—	0.4	53.3
June-SoilB		Full-bloom	91.8	0.7	204.4	17.9	34.7	7.7	60.3	50.0
July-Spot	18 October	Full-bloom	136.8	0.3	439.9	10.8	14.0	13.1	38.0	14.3
July-SoilB	2 December	Full-mature	135.6	4.1	7413.8	272.4	356.2	637.3	1265.9	4.3
August-SoilB		Early seed mature	112.0	2.5	3028.6	151.3	144.5	118.0	413.7	0
(B) 2022										
Establishment Method	Harvest Date	Maturity at Harvest	Plant Height (cm)	Plant Density (m ^{-2.2})	Fresh Yield (g m ⁻²)	Dry Matter Yield (g m ⁻²)				Lodging by Typhoon (%)
						Leaves	Stems	Inflore-Scences	Whole Plants	
April-TransP	26 July	Full-mature	151.8 ± 3.8 td	11.1 ± 0.0 a	7707.4 ± 1636.4 ab	213.3 ± 52.5 bc	356.7 ± 61.5 c	1042.7 ± 222.2 a	1612.6 ± 335.5 a	0
April-SoilB		Full-mature	177.7 ± 0.7 c	11.1 ± 0.0 a	11,023.7 ± 2323.7 a	355.3 ± 67.8 a	624.6 ± 89.3 b	828.5 ± 223.5 a	2043.8 ± 270.2 a	0
May-TransP	25 August	Full-mature	207.7 ± 6.7 b	11.1 ± 0.0 a	8180.7 ± 243.9 ab	130.7 ± 4.9 cd	711.5 ± 51.6 ab	618.5 ± 41.7 a	1460.7 ± 16.9 a	0
May-SoilB		Full-mature	231.5 ± 9.7 a	11.1 ± 0.0 a	11,464.8 ± 2232.5 a	306.3 ± 37.0 ab	903.4 ± 121.9 a	645.4 ± 119.9 a	1685.3 ± 286.8 a	0
August-TransP	4 November	Full-bloom	209.3 ± 11.3 b	5.8 ± 1.1 b	4022.9 ± 717.2 bc	85.6 ± 13.9 d	278.2 ± 8.2 c	103.7 ± 22.5 b	467.5 ± 40.8 b	46.1
August-SoilB		Full-bloom	144.0 ± 5.2 d	5.3 ± 0.4 b	2313.0 ± 93.2 c	71.8 ± 6.1 d	156.5 ± 10.1 c	77.7 ± 15.0 b	306.0 ± 25.4 b	47.3
(C) 2023										
Establishment Method	HarvestDate	Maturity at Harvest	Plant Height (cm)	Plant Density (m ⁻²)	Fresh Yield (g m ⁻²)	Dry Matter Yield (g m ⁻²)				Lodging by Typhoon (%)
						Leaves	Stems	Inflore-Scences	Whole Plants	
April-TransP	10 July	Full-mature	177.2 ± 8.3 b	11.1 ± 0.0 ns	8965.0 ± 303.3 c	259.2 ± 8.6 c	383.1 ± 29.8 c	716.1 ± 22.9 a	1358.4 ± 60.0 c	0
April-SoilB	22 July	Full-mature	190.7 ± 7.4 b	11.1 ± 0.0	10,973.9 ± 601.6 abc	355.2 ± 21.0 b	657.6 ± 56.7 b	881.8 ± 91.0 a	1894.7 ± 164.7 a	0
May-TransP	12 August	Full-mature	205.7 ± 7.1 ab	10.7 ± 0.3	9059.6 ± 450.4 bc	279.4 ± 19.8 c	548.4 ± 31.8 b	477.7 ± 22.3 b	1305.6 ± 46.8 c	4.9
May-SoilB	26 August	Full-mature	223.8 ± 8.4 a	10.6 ± 0.2	12,090.7 ± 92.9 a	376.7 ± 23.3 b	904.8 ± 14.8 a	231.1 ± 14.7 c	1512.6 ± 31.0 bc	3.5
August-TransP	11 November	Full-mature	220.3 ± 9.1 a	10.8 ± 0.2	10,696.7 ± 945.2 abc	377.1 ± 11.0 b	616.7 ± 50.1 b	761.7 ± 94.5 a	1755.6 ± 151.6 ab	0
August-SoilB	11 November	Full-mature	217.2 ± 3.5 a	10.9 ± 0.1	11,039.4 ± 839.5 ab	465.3 ± 11.6 a	631.6 ± 55.3 b	322.7 ± 56.7 bc	1419.7 ± 122.1 bc	0

Note: † mean ± standard error, n = 3. Values followed by the same letter within the column are not significantly different between growing seasons at the 5% level of probability by the least significant difference. ns: $p > 0.05$.

3.5. Herbage Quality of In Vitro Digestibility of DM and CP Content in 2022

The IVDMD and CP content in each plant fraction at the time of harvest in 2022 are shown in Table 3. Across methods and months of establishment, both IVDMD and CP contents tended to be higher in the inflorescences and leaves than in the stems, leading to a higher IVDMD above 63% and a CP content above 10.5% DM in whole plants, except for the lower values of IVDMD and CP contents from the establishment in May. The highest IVDMD and CP contents from the establishment in August were reflected in the suppressed growth by typhoon damage in these plots. Establishment methods marginally affected IVDMD and CP content, while these two quality attributes tended to be higher in soil seed balls than in transplants for IVDMD established in April and August and CP content in August. Therefore, the combination of forage quantity and quality appeared to be the best in the soil seed ball plot established in April.

3.6. Silage Quality in 2021–2023

The fermentation quality of amaranth silage was evaluated for DM percentage, pH, lactic acid, acetic acid, and propionic acid, ranging from 19.29 to 24.30, 4.52 to 6.39, 1.25 to 9.91 g kg⁻¹ DM, 6.56 to 23.69 g kg⁻¹ DM, and 1.56 to 3.53 g kg⁻¹ DM, respectively, while negligible butyric acid and valeric acid were produced, ranging from 0 to 0.23 g kg⁻¹ DM and 0.24 to 5.66 g kg⁻¹ DM, respectively (Table 4). Furthermore, the VBN production was negligible, ranging from 0.06 to 0.36 g kg⁻¹ DM, and the ratio of VBN/TN remained at a low level of 0.23% to 1.28% (Table 4).

Table 3. Dry matter digestibility in vitro (IVDMD) and crude protein (CP) content in a range of establishment methods in 2022.

Treatment for Establishment	IVDMD (%)				CP (% DM)			
	Leaves	Stems	Inflore-Scences	Whole Plants	Leaves	Stems	Inflore-Scences	Whole Plants
April-TransP	63.95 ± 0.83 †e	48.74 ± 0.43 b	67.70 ± 0.66 bc	62.97 ± 0.57 a	13.81 ± 0.99 b	3.24 ± 0.70 b	15.41 ± 0.47 bc	12.47 ± 0.63 bc
April-SoilB	70.82 ± 0.62 bc	48.47 ± 2.42 b	71.47 ± 1.37 ab	63.08 ± 1.70 a	15.63 ± 1.29 b	4.06 ± 1.33 b	13.38 ± 1.00 c	10.53 ± 1.26 cd
May-TransP	66.52 ± 0.79 de	43.37 ± 1.00 c	60.99 ± 1.84 cd	53.27 ± 0.20 b	9.10 ± 1.02 c	2.64 ± 0.58 b	13.82 ± 1.85 c	7.59 ± 1.23 d
May-SoilB	68.33 ± 0.89 cd	37.96 ± 1.18 d	59.02 ± 1.14 d	49.64 ± 1.48 b	15.94 ± 2.25 b	4.66 ± 1.46 b	14.15 ± 1.71 c	9.72 ± 1.80 cd
August-TransP	73.69 ± 1.99 b	54.99 ± 1.41 a	78.12 ± 3.64 a	63.10 ± 1.36 a	22.99 ± 1.36 a	11.44 ± 0.87 a	19.15 ± 1.78 ab	15.15 ± 1.33 ab
August-SoilB	77.97 ± 0.54 a	56.30 ± 0.64 a	75.28 ± 3.22 a	66.15 ± 1.21 a	24.88 ± 0.71 a	13.41 ± 0.98 a	22.30 ± 0.41 a	18.36 ± 0.80 a

Note: † mean ± standard error, n = 3. Values followed by the same letter within the column are not significantly different between growing seasons at the 5% level of probability by the least significant difference.

Table 4. Effects of seasons and establishment methods on the quality attributes of amaranth silage in the 2021–2023 seasons.

Established Month and Year	July 2021	August 2022		August 2023	
Method of Establishment	Soil Seed Balls	Soil Seed Balls	Transplants	Soil Seed Balls	Transplants
n	2	3	3	3	3
Percentage of dry matter (DM)	17.07 ± 0.00 b†	Wilted forages 25.78 ± 1.75 a	21.95 ± 4.54 ab	19.37 ± 0.79 ab	24.59 ± 1.19 a
Percentage of dry matter (DM)	19.29 ± 0.22 c	Silage quality attribute 23.30 ± 0.44 a	22.81 ± 0.47 ab	20.01 ± 0.79 bc	24.30 ± 1.56 a
pH	6.39 ± 0.22 a	5.58 ± 0.33 b	5.53 ± 0.05 b	4.52 ± 0.04 c	4.99 ± 0.23 bc
Lactic acid (g kg ^{−1} DM)	9.91 ± 4.09 a	3.10 ± 0.39 b	3.09 ± 0.49 b	1.25 ± 0.16 b	2.00 ± 0.70 b
Acetic acid (g kg ^{−1} DM)	6.56 ± 0.01 c	23.69 ± 1.99 a	16.59 ± 0.49 b	10.43 ± 0.81 c	8.35 ± 1.53 c
Propionic acid (g kg ^{−1} DM)	2.49 ± 0.73 ns	1.56 ± 0.33	3.09 ± 0.11	2.04 ± 0.60	3.53 ± 0.85
Butyric acid (g kg ^{−1} DM)	0.00 ± 0.00 ns	0.13 ± 0.06	0.23 ± 0.13	0.00 ± 0.00	0.00 ± 0.00
Valeric acid (g kg ^{−1} DM)	0.64 ± 0.14 b	0.24 ± 0.12 b	0.49 ± 0.09 b	5.66 ± 0.31 a	5.07 ± 0.23 a
Volatile basic nitrogen (VBN, g kg ^{−1} DM)	0.06 ± 0.06 c	0.36 ± 0.02 a	0.34 ± 0.01 a	0.17 ± 0.01 b	0.11 ± 0.01 bc
Total nitrogen (TN, g kg ^{−1} DM)	23.32 ± 1.97 ns	28.71 ± 1.94	26.43 ± 0.88	29.71 ± 3.00	29.71 ± 2.21
VBN/TN (%)	0.23 ± 0.23 c	1.27 ± 0.06 a	1.28 ± 0.02 a	0.58 ± 0.04 b	0.47 ± 0.01 b

Note: † mean ± standard error, n = 3, except for July 2021 (n = 2). Values followed by the same letter within the column are not significantly different between growing seasons and methods at the 5% level of probability by the least significant difference. ns: $p > 0.05$.

4. Discussion

4.1. Effect of Seasons and Establishment Methods on Establishment and Growth of Amaranth

We observed a large difference in the establishment rate between direct seeding and pretreatment due to poor establishment methods in April and May 2021, affected by continuous heavy rainfall from mid-May to mid-June above 100 mm of 10-day rainfall (Figure 1A), which tends to increase the frequency influenced by global climate change [1]. Since amaranth is used to growing in a water-deficient area [7], the species is an acceptable drought-tolerant forage [6], while, in the present study, amaranth is considered as highly sensitive to water-lodged conditions. Therefore, in southern Kyushu, located in humid temperate warm regions, pretreatment methods are recommended for the safe and stable establishment of amaranth. Compared to soil seed balls and transplants, soil seed balls were shown to be nearly equal in establishment rate (Figure 2B), changes in shoot density (Figure 3B,C), and plant height (Figure 4B,C). Therefore, a less intensive and cost-effective pretreatment method for soil seed balls is recommended for the establishment of amaranth in the region.

Baturaygil et al. [17] examined the change in phenotypic attributes in ten amaranth genotypes in a temperate elevated area in Germany, where the final plant height at 135 days after sowing ranged from 160 to 250 cm, except for a genotype “Bärnkrafft”, which corresponds to the present plant height at harvest in 2022 (Figure 4B) and in 2023 (Figure 4C). The increasing rates of plant height were higher in the May plots in both years and in the August plots in 2023 than in Germany [17], which was affected by a monthly average temperature below 21 °C compared to above 25 °C from July to September in the present study (Figure 1). Amaranth grown in the short-day period when sown in July and August, produced similar dry matter as in the long-day period sown in May if the plant density was guaranteed to escape from typhoon damage, as shown in Table 2C. Therefore, it is estimated that the amaranth cultivar examined in the present study can possess enough of a vegetative phase, even when it is established in August under a short-day photoperiod [17].

4.2. Effect of Seasons and Establishment Methods on Amaranth Yield, Herbage Quality, and Silage Fermentation Quality

DM yields were variable between established years, while they were lower in 2021 than in 2022 and 2023, when the soil seed ball plots showed higher or almost similar DM yields when compared to in transplants (Table 2). The level of DM production in the plots established in April 2022 and 2023 was higher at 16.1–20.4 t ha⁻¹ and 13.6–18.9 t ha⁻¹ (Table 2B,C, respectively) than in sole amaranth plots under an excellent drip irrigation system at 11.52 t ha⁻¹ in Iran [6]. However, under typhoon damage, the current DM yield of amaranth was lower than plots under a less effective irrigation system in Iran [5].

By the regression line between CGR and days after establishment (Figure 5), it is estimated that the plant growth rate started to increase linearly around 40 days after establishment, which was almost correlated with the stage of initial stem elongation. The highest CGR at around 50 g m⁻² day⁻¹ approached the record of efficient C₄ maize crop, since amaranth species are dicotyledonous C₄ plants [7].

The quality attributes of amaranth forage in IVDMD and CP content over three months of establishment and two pretreatments in 2022 were highest in plots established in August harvested at the full-bloom stage, followed by those in April and May at the fully mature stage (Table 3), corresponding to [5], which describes the ontogenetic change in amaranth CP content from 10.59% DM in the initial bloom stage to 9.49% DM in the mature stage, and with the data [21], which describes a decrease in CP content from 12.52% DM in the early-bloom stage to 11.51% DM in the heading stage. In the present study, whole-plant IVDMD decreased dramatically from 63.1–66.2% in August plots to 49.6–53.3% in May plots due to the severe decrease in stem IVDMD with ontogenetic changes (Table 3). Changes in the digestibility of DM are suggested for the concomitant increase in acid detergent fiber content (ADF) from 34.08% DM in the initial bloom stage to 40.27% DM in the mature stage [5] since a linear negative regression was obtained between the ADF content (x) and

IVDMD (y) in all crops and fractions of plants ($y = -0.987x + 96.30$, $r = -0.867$, $p < 0.01$) in 2022 [Unpublished]. Taking consideration of the whole of the establishment, DM yield, and forage quality attributes, the best combination for months and methods of establishment in amaranth was recommended for soil seed balls established in April, which were recorded in 2022 and 2023.

In the present study, the moisture content of the silage was favorable, ranging from 75.70% to 80.71% FM (Table 4), did not differ between seasons and methods in the present study, and was almost the same as the maturity stage at 77.23% FM [5] and the mid-maturity stage at 81.3% in the variety 'Maria' [7]. In the present study, no additives were incorporated for silage processing; however, some improvements in amaranth silage processing were reported with the addition of starter lactic acid bacteria (LAB) [30], with the addition of LAB, glucose, and formic acid after pre-drying to reduce the moisture content by 65% [20], and with the addition of LAB, *Lactobacillus plantarum*, cellulase, and their combination, which could increase the quality of silage as a raw material of high-moisture amaranth with rice straw [31]. Therefore, in the present study, the pH values did not decrease as much, ranging from 4.52 to 6.39, and the lactic acid content was very low, in a range from 1.25 to 9.91 g kg⁻¹ DM (Table 4). However, with the addition of an LAB content of 10⁸ CFU/mL at a rate of 100 mL per 1.0 kg of amaranth FM, the pH of the silage decreased quite dramatically to 4.2 within 10 days after ensilage and the lactic acid content of the silage increased to 75.1% of the total organic acid content compared to a slow decrease in pH above 6.0 and a lower ratio of lactic acid content at 29.1% in the control without treatment [30]. The silage of tropical C₄ grasses such as guineagrass (*Panicum maximum* Jacq.) had a high moisture content above 75% FM, and the decrease in pH after ensilage was slow, at around 5.8 without additives, while, in the guineagrass silage with glucose addition at 1% FM, the pH declined sharply to below 5.0 and the lactic acid content increased sharply above 2% DM within 10 days after ensilage compared to negligible lactic acid production without additives [32]. Based on the overall samples in Table 4, the pH was maintained as high above 4.5, and the formation of organic acids was primarily of the acetic acid type, while the production of butyric and valeric acids was suppressed and the VBN/TN ratio was less than 1.28%, showing the typical favorable silage processing for C₄ forage species [32].

5. Conclusions

The present study revealed how to solve the sensitivity of amaranth to typhoon damage by changing sowing seasons and improving established methods in humid and warm regions of Japan. Taking consideration of the whole of the establishment, DM yield, and forage quality attributes, the best combination for seasons and methods was presented as the soil seed ball method, established in April. The pre-dried amaranth silage revealed that the amaranth silage tended to have a high pH above 4.5, and the formation of organic acids was of the acetic acid type, while the production of butyric and valeric acids was suppressed and the VBN/TN ratio was less than 1.28%, which shows the typical favorable silage processing for the C₄ forage species.

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