

Comparative physiological activity and productivity of two local West Timor (Indonesia) maize in response to *t'sen*, row, and monoculture cropping patterns

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Abstract. Dimu-Heo YH, Indradewa D, Putra ETS, Purwanto BH. 2024. Comparative physiological activity and productivity of two local West Timor (Indonesia) maize in response to *t'sen*, row, and monoculture cropping patterns. *Biodiversitas* 25: 1718-1728. *T'sen* is a traditional cropping pattern practiced in West Timor, where farmers plant maize, cowpeas, and pumpkins together in one hole. Field research was carried out to compare the productivity and physiological activity of maize grown in monoculture, *t'sen*, and row cropping patterns. The research followed a split-plot design with four replications. The main plot consisted of monoculture, *t'sen*, and row cropping patterns, while the subplots included local maize varieties: Kupang and TTS. The results showed that the *t'sen* cropping pattern did not cause any differences in stomatal activity, chlorophyll properties, transpiration rate, and photosynthesis rate, resulting in productivity that was not significantly different from monoculture. In contrast, the row cropping pattern led to an increase in the width of the stomatal opening but caused a decrease in the rate of transpiration and photosynthesis, resulting in lower productivity compared to monoculture. Additionally, the research found the productivity of the Kupang variety was higher than the TTS variety. Furthermore, both cropping patterns resulted in higher total protein yields due to cowpea and pumpkin fruit production compared to monoculture, with the *t'sen* showing significantly higher than the row cropping pattern.

Keywords: Maize, physiological activities, productivity, row cropping pattern, *t'sen* cropping pattern

Abbreviations: M: maize monoculture; T'sen: maize + cowpea + pumpkin in *t'sen* cropping pattern; Rows: maize + cowpea + pumpkin in row cropping pattern; PR: photosynthetic rate; TR: transpiration rate; SW: width of stomata; SD: stomatal density; Chl: chlorophyll content of leaves; PC: protein content; productivity: yield per hectare

INTRODUCTION

T'sen, or *t'sen tabua bola mese*, is a local wisdom farming practice in the West Timor where maize, cowpeas, and pumpkins are planted together in a single hole. According to Palaniappan (1985), Teshome (2019), and Maitra et al. (2021), planting a mixture of two or more different types of plants simultaneously or single plants sequentially on a unit of land is called intercropping. Several familiar forms of intercropping planting patterns generally include mixed, row, strip, and relay intercropping. This means that the *t'sen* cropping pattern is a typical and extreme form of intercropping because the three plant types are planted simultaneously in the same planting hole.

Traditionally, the *t'sen* cropping pattern was developed by West Timorese farmers to reduce the risk of failure, especially due to the marginal condition of agricultural land, and to increase the diversity and security of food (Dimu-Heo et al. 2022). Basuki and de-Rosari (2017); Matheus et al. (2017) have noted that West Timor's agricultural land generally lacks nutrients and organic

matter, has less stable soil aggregates that are prone to erosion, and experiences limited water availability due to the short and frequent rainy season, categorizing it as marginal land. This is in line with previous reports that traditionally, intercropping is carried out by small and marginal farmers to hindering the risk of crop failure, increase diversity, ensure yield stability, and enhance food security through more efficient land use, resource utilization, and limited labor utilization. It is also seen as approach to sustainable agricultural cultivation (Qamar-Uz-Zaman and Malik 2000; Yin et al. 2020; Maitra et al. 2021). In addition to these objectives and advantages, intercropping enhances plant competition for growth resources such as light, water, nutrients, and growing space. This competition may reduce crop yield, but other plants' yield in the intercropping system typically offsets this decrease. Intercropping can also impact the micro-environmental conditions of the cropping system, such as light intensity at the soil surface due to light absorption by a denser canopy, which affects soil temperature and moisture content. Additionally, the plant litter that falls decomposition to the soil surface can influence nutrient

availability. Several studies have reported a decrease in light intensity at the soil surface, an increase in soil moisture content, and enhanced nutrient uptake in intercropping compared to monoculture (Wang et al. 2014; Wang et al. 2015; Cong et al. 2015; Romaneckas et al. 2020; Zhu et al. 2022; Nasar et al. 2023). Other studies have reported that the influence can vary depending on the cropping pattern and type of crops used (Zhang et al. 2014; Singh et al. 2023; Wang et al. 2023).

The dynamics of the reciprocal relationship between plants and the environment in intercropping patterns will influence physiological activities and ultimately affect the productivity of each intercropping plant. Previous studies have shown differences in physiological responses and plant productivity in intercropping compared to monoculture; intercropping results in an increase in stomata opening width and density, chlorophyll content, transpiration rate, and maize photosynthesis, leading to significantly higher productivity than monoculture (Filho 2000; Chen et al. 2020; Yang et al. 2022). In contrast, Li et al. (2020) found that stomata activity, chlorophyll content, transpiration and photosynthesis rates, and maize productivity were lower in intercropping compared to monoculture. However, to the best of our knowledge, no research has been conducted on the physiological activity and productivity of maize in *t'sen* cropping patterns. Therefore, this research aims to determine physiological activity and its influence on maize productivity in the *t'sen* cropping pattern and compare it to row cropping patterns and monoculture. This research result is expected to provide a theoretical framework for developing *t'sen* cropping patterns and utilizing marginal land.

MATERIALS AND METHODS

Plant materials

The research used two local West Timor maize varieties from Kupang and South-central Timor (TTS). Both varieties are high productivity, but the Kupang variety

experiences a lower decrease in productivity than the TTS variety when planted in the *t'sen* cropping pattern based on previous research (Dimu-Heo et al. 2022). The cowpea and pumpkin varieties used were from each district, and the cowpea variety used is a variety with a creeping growth type so that during its growth, it will creep on the maize stalks.

Research site and design

The research was conducted at the demonstration land of the Agricultural Extension Center, Patuk Sub-district, Gunung Kidul District, Yogyakarta Province, Indonesia, from December 2020 to May 2021. The research site is located at coordinates 7°52'15"S and 110°31'12" E (Figure 1), with an elevation of 125 meters above sea level.

The research used a split-plot design with four replications. The main plot consists of all the cropping patterns, which includes planting maize monoculture (Monoculture), planting maize, cowpeas, and yellow pumpkin in one planting (*t'sen* cropping pattern), and planting maize, cowpeas, and yellow pumpkin in separate rows (row pattern cropping). The sub-plot included the local maize variety of Kupang (Kupang) and the local maize variety of South-central Timor (TTS). The experimental plot measured 24 m² (4 × 6m) with a distance of 1.5 m between plots and 2.5 m between blocks. The maize planting distance was 75 × 100 cm for all cropping pattern treatments. In the *t'sen* cropping pattern, cowpeas and pumpkins were planted in the same hole by the maize plants, while in the row cropping pattern, the cowpeas and pumpkins were each planted 25 cm to the left and right of the maize plants, forming a row of the two plants (Figure 2). The total number of plants per plot is 49 for monoculture and 147 for the *t'sen* and row planting pattern. Cowpea and pumpkin monocultures were not included in the experimental design, as both crops are typically planted with maize by farmers in West Timor. The research focused on the physiological activity of maize plants and its impact on maize productivity in each cropping pattern.

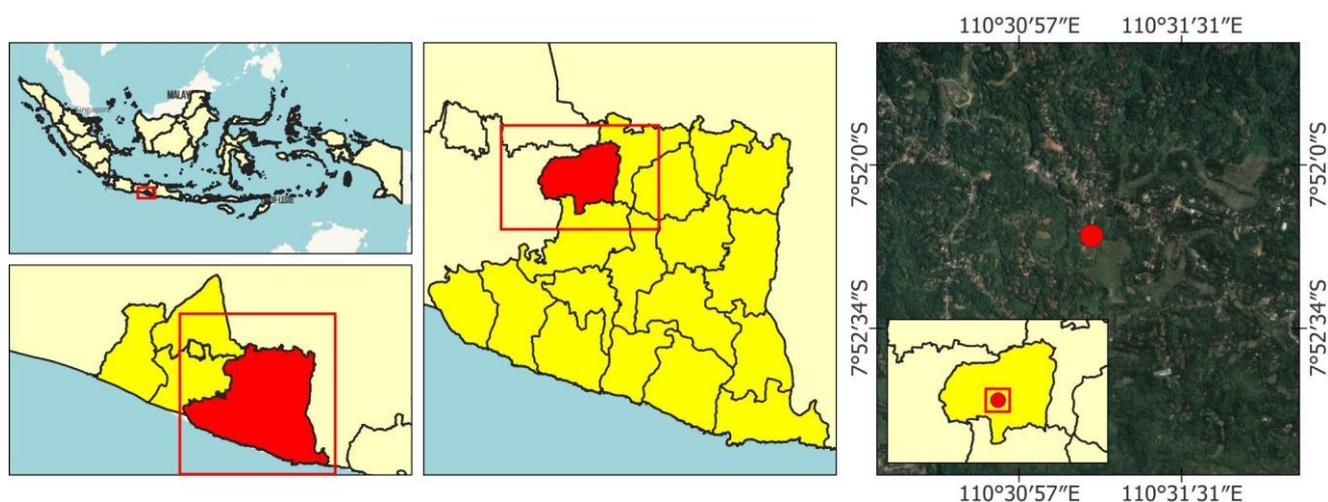


Figure 1. Geographical locations of the study area on the experimental field of the Agricultural Extension Center (BPP) Patuk, Gunung Kidul, Yogyakarta, Indonesia

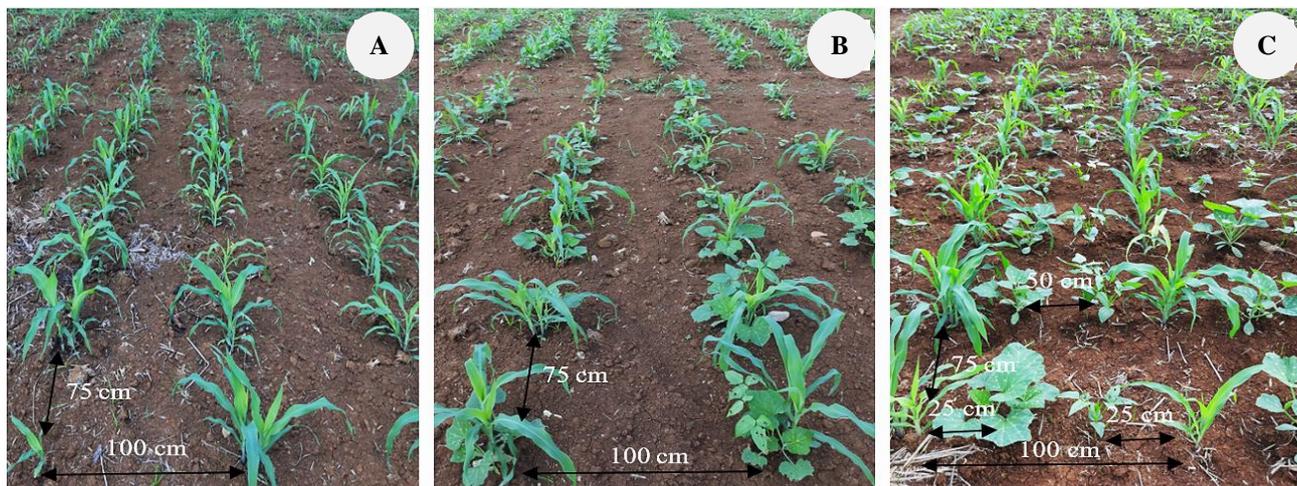


Figure 2. Maize cropping pattern in the experiment design: maize monoculture (A), *T'sen* cropping pattern: maize + cowpea + pumpkin in one planting hole (B), row cropping pattern: maize + cowpea + pumpkin in separate rows (C)

According to the treatment protocol, two seeds of each plant type are placed in a planting hole. When the plants are grown two weeks old, thinning is carried out by leaving one plant of each type in each planting hole. The plants are watered once after planting and rely solely on rain for irrigation, and no fertilization is used during the planting period. These conditions replicate those used by farmers in West Timor, where no fertilization is used, and irrigation is dependent on rainfall.

Observation

Environmental variables of the research site

The physical and chemical properties of soil samples from the research area were analyzed. Weekly microclimate observations, including temperature, humidity, and number of rainy days were recorded at the research site starting when the plants were 3-14 weeks after planting (WAP). Observations of soil moisture content and N, P, and K nutrient uptake were carried out 9 WAP, marking the conclusion of the vegetative growth phase.

Plant physiological and productivity

Physiological observations were also carried out in 9 WAP. The observations included the density and width of stomata, chlorophyll a and b content, total and ratio of chlorophyll a to b, and transpiration rate and photosynthesis. The photosynthesis and transpiration rates were measured using the LICOR LI-6400 photosynthetic analyzer. Measurements were taken on two sample plants in each plot, with two readings on fully opened leaves at the middle and top of the plant. The total chlorophyll content was analyzed spectrophotometrically using the method described by Hendry and Price (1993). Maize productivity was assessed after harvest, including maize grains' productivity and protein content. The maize and cowpea grains were weighed after drying until they reached a moisture content of 12%, and the grain moisture content was measured using a grain moisture meter JV 010S series. The protein content of each plant type is calculated by multiplying its productivity by its protein content. Total protein productivity is determined by summing up the

protein productivity of each plant in the planting pattern. The productivity data and protein content of cowpeas and pumpkins were used as additional data to calculate the total protein yield of the *t'sen* and the row cropping patterns.

Data analysis

All the physiological maize activities and yield observations were collected and statistically analyzed using a two-way Analysis of Variance (ANOVA). Tukey's HSD was used to assess the mean differences. The Pearson correlation was used to identify the relationship between the observed variables. All analyses were performed in SAS 9.4. The evaluation of the relationship between the variables was based on the correlation coefficient value as described by Evans (1996): 0-0.19 (very weak relationship); 0.2-0.39 (weak cohesiveness); 0.4-0.59 (medium close relationship); 0.6-0.79 (strong relationship); and 0.8-1 (very strong relationship). A positive correlation coefficient indicates a synergistic relationship, while a negative correlation coefficient indicates an antagonistic relationship. The data for cowpea and pumpkin were not analyzed for variance and were presented as supplementary data.

RESULTS AND DISCUSSION

Soil characteristics, environment microclimate, and nutrient uptake of maize

The initial soil characteristics in this study, as shown in Table 1, included a clay loam texture, slightly acidic pH, high cation exchange capacity (CEC), low organic carbon content, low total nitrogen content, medium available phosphorus, low exchangeable potassium, moderate exchangeable sodium, very high exchangeable calcium, and high available magnesium. Figure 3 shows the average weekly microclimate conditions observed during the study, including morning, noon, and afternoon air temperatures of 28.9, 35.5, and 30.8°C, respectively, with corresponding air humidity levels of 76.0, 64.7, and 69.0%. On average, there were 2.1 rainy days per week.

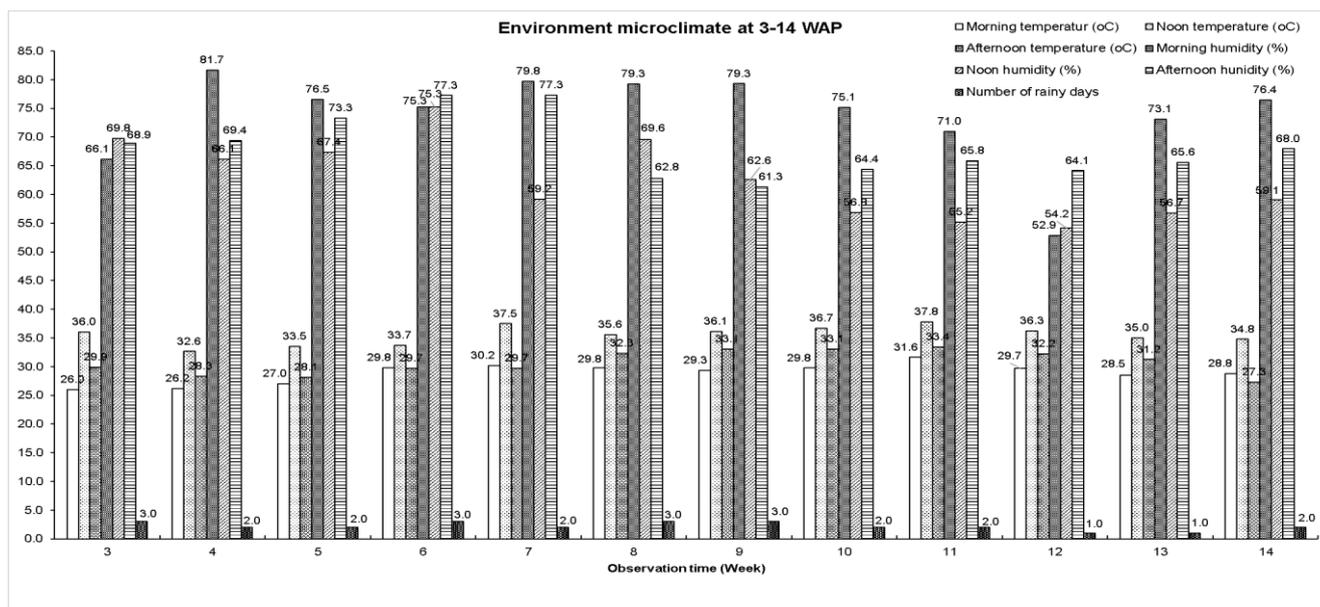


Figure 3. The average weekly microclimate conditions at 3-14 Week After Planted (WAP)

Table 1. Initial soil characteristic of the research site

Physical characteristic						Chemical characteristic					
Sand (%)	Silt (%)	Clay (%)	pH (H ₂ O)	CEC (cmol ⁽⁺⁾ kg ⁻¹)	Organic carbon (%)	N (%)	P (ppm)	K (cmol ⁽⁺⁾ kg ⁻¹)	Na (cmol ⁽⁺⁾ kg ⁻¹)	Ca (cmol ⁽⁺⁾ kg ⁻¹)	Mg (cmol ⁽⁺⁾ kg ⁻¹)
27.92	33.67	38.41	5.9	57.23	1.64	0.13	13	0.43	0.54	22.83	6.82

Table 2. Soil moisture content and N, P, and K nutrient uptake of maize as affected by cropping patterns and varieties

Treatment	Soil moisture content (%)	Nutrient uptake (%) of maize		
		N	P	K
Cropping patterns				
Maize monoculture	20.91 ^b	1.52 ^b	0.23 ^a	1.26 ^a
<i>T'sen</i> M+C+P	28.71 ^a	1.69 ^{ab}	0.20 ^a	1.12 ^a
Row M+C+P	29.95 ^a	1.72 ^a	0.19 ^a	1.14 ^a
Varieties				
Kupang	26.74 ^a	1.65 ^a	0.21 ^a	1.15 ^a
TTS	26.31 ^a	1.65 ^a	0.20 ^a	1.20 ^a

Note: The same letter in each column was not significantly different based on Tukey's test (p<0.05)

Table 2 presents the soil moisture content and N, P, and K nutrient uptake of maize, which are affected by cropping patterns and varieties. The table indicates no interaction between cropping patterns and varieties on the soil moisture content and maize plants' N, P, and K nutrient uptake. Cropping patterns and varieties result in different responses to soil moisture content and N, P, and K nutrient uptake.

Cropping patterns significantly influenced soil moisture content, with *t'sen* and row cropping patterns increasing it by 37.3 and 43.2%, respectively, compared to monoculture. There was no significant difference in soil moisture content between the two intercropping patterns. Meanwhile,

planting Kupang and TTS varieties did not significantly affect soil moisture content.

Cropping patterns significantly influenced N nutrient uptake. Intercropping with *t'sen* and row cropping patterns led to varying increases in N nutrient uptake. In the *t'sen* cropping pattern, the increase in N nutrient uptake did not differ from monoculture and row cropping patterns. However, in the row cropping pattern, N uptake increased by 13.2% compared to monoculture. The research results also showed that planting with Kupang and TTS varieties did not show significant differences in N nutrient uptake levels.

Different conditions occur in the absorption of P and K nutrients. Intercropping with *t'sen* and row cropping patterns did not cause differences in P and K nutrient absorption compared to monoculture, although there was a tendency to decrease it. Meanwhile, the Kupang and TTS varieties showed no significant differences in P and K nutrient uptake.

Physiological activities and productivity of maize under *t'sen*, row, and monoculture cropping pattern

Physiological activities

The results showed that cropping patterns and varieties influenced maize's physiological activity differently. Stomatal density, opening width, and leaf chlorophyll were not influenced by the interaction of cropping pattern and variety (Table 3), but there were different influences from cropping pattern and variety. Meanwhile, the research also

shows that transpiration and photosynthesis rates were influenced by the interaction of cropping patterns and varieties (Figure 4).

The results indicated that cropping patterns and varieties affected maize's physiological activity differently. Stomatal density, opening width, and leaf chlorophyll were not affected by the interaction of cropping pattern and variety (Table 3), but there were distinct effects from cropping pattern and variety. The study also revealed that transpiration and photosynthesis rates were influenced by the interaction of cropping patterns and varieties (Figure 4).

Table 3 shows no differences in stomatal density between *t'sen* and row cropping patterns compared to monoculture. However, there is a notable differences in stomatal density between the Kupang and TTS variety having a significantly higher stomatal density. Additionally, cropping patterns have a significant effect on the width of the stomatal opening, with both *t'sen* and row cropping patterns reducing the width of the stomatal opening compared to monoculture. The *t'sen* cropping pattern a smaller decrease in width compared to the row cropping pattern resulting in a significantly smaller stomatal opening width.

The research findings indicated that cropping patterns and varieties have no impact on the chlorophyll a and b content of maize leaves. The *t'sen* cropping pattern showed no significant differences in chlorophyll a and b content compared to monoculture and row cropping patterns. The chlorophyll a to b ratio remained consistent across all cropping patterns, indicating similar total chlorophyll content. The total chlorophyll content and the ratio of chlorophyll a to b had similar responses across all cropping patterns. Additionally, there were no significant differences in chlorophyll a, chlorophyll b, total chlorophyll, and the ratio of chlorophyll a to b between the Kupang and TTS varieties.

Figure 4.A illustrates that intercropping in *t'sen* and row cropping patterns results in different responses in transpiration rate between the two varieties compared to monoculture. The transpiration rate of the Kupang variety was not affected, but the cropping patterns influenced the TTS variety. The TTS variety exhibited a lower transpiration rate in row cropping patterns than in monoculture, whereas there was no significant difference in

the *t'sen* cropping pattern. In the *t'sen* cropping pattern, the transpiration rate of the TTS variety did not differ from the row cropping pattern. Figure 3.A also indicates no difference in transpiration rates between the Kupang and TTS varieties in monoculture and *t'sen* cropping patterns.

Figure 4.B illustrates that intercropping with *t'sen* and row cropping patterns causes different responses in photosynthesis rates between the two varieties compared to monoculture. The photosynthesis rate of the Kupang variety was affected, while the cropping patterns did not influence the TTS variety. In the row cropping pattern, the Kupang variety exhibited a lower photosynthesis rate than in monoculture, whereas, in the *t'sen* cropping pattern, there was no significant difference. In the *t'sen* cropping pattern, the photosynthesis rate of the TTS variety did not differ from planting in the row cropping pattern. Figure 3.B also indicates no difference in photosynthesis rates between the Kupang and TTS varieties in monoculture and *t'sen* cropping patterns.

Productivity, protein content, and protein yield of maize, cowpeas grains, and pumpkin fruits difference.

The productivity, protein yield of maize grains, and total protein yield in monoculture *t'sen*, and row cropping patterns are shown in Table 4, while the protein content of maize grains is show in Figure 5. Table 4 shows no interaction between cropping patterns and varieties on maize productivity, but both treatments affect maize productivity. Maize productivity decreases in both *t'sen* and row cropping patterns, with the decrease being insignificant in the *t'sen* cropping patterns, resulting in similar productivity to monoculture. There is no significant difference in productivity between *t'sen* and row cropping patterns. The research also shows that the Kupang variety has higher productivity than the TTS variety. Meanwhile, Figure 5 illustrates an interaction effect between cropping patterns and variety on the protein content of maize grains. The cropping pattern does not affect the grain protein content of the Kupang and TTS varieties. However, the TTS variety, which has a lower grain protein content than the Kupang variety in monoculture conditions, planted in *t'sen* and row cropping patterns increased grain protein content which is no different from the Kupang variety.

Table 3. Stomatal density, stomatal opening width, and leaf chlorophyll of maize as affected by cropping patterns and varieties

Treatment	Stomatal density (pore μm^2)	Stomatal opening width (μm)	Leaf chlorophyll (mg.g ⁻¹)			
			Chlorophyll a	Chlorophyll b	Total chlorophyll	The ratio of chlorophyll a to b
Cropping patterns						
Maize monoculture	91.00 ^a	3.73 ^a	0.58 ^a	0.45 ^a	1.02 ^a	1.34 ^a
<i>T'sen</i> M+C+P	86.88 ^a	3.67 ^a	0.48 ^a	0.40 ^a	0.88 ^a	1.19 ^a
Row M+C+P	87.13 ^a	3.30 ^b	0.58 ^a	0.45 ^a	1.03 ^a	1.28 ^a
Varieties						
Kupang	93.74 ^a	3.51 ^a	0.55 ^a	0.43 ^a	0.98 ^a	1.27 ^a
TTS	82.93 ^b	3.63 ^a	0.54 ^a	0.43 ^a	0.97 ^a	1.26 ^a

Note: The same letter in each column was not significantly different based on Tukey's test ($p < 0.05$)

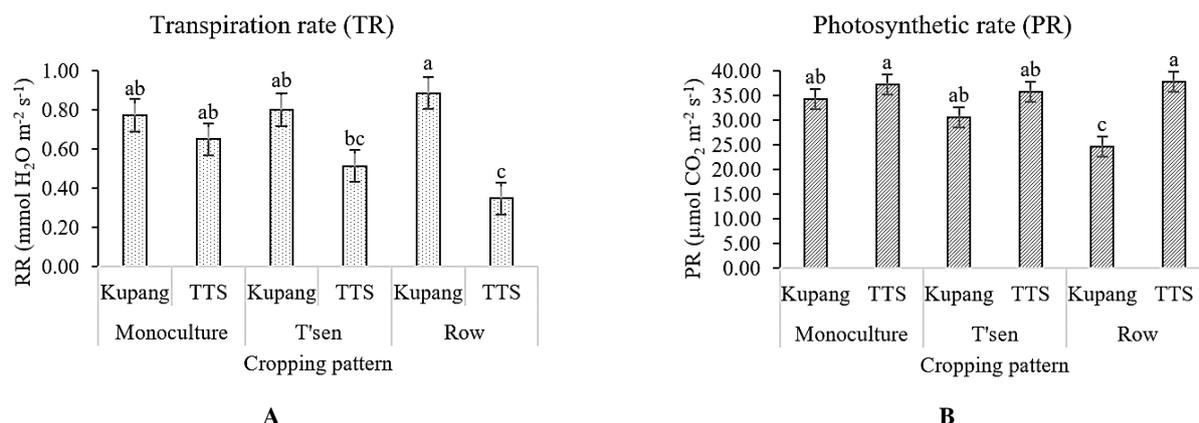


Figure 4. The rate of transpiration (A) and photosynthetic (B) of maize are affected by cropping patterns and varieties. The same letters in each chart were not significantly different based on Tukey's test ($p < 0.05$)

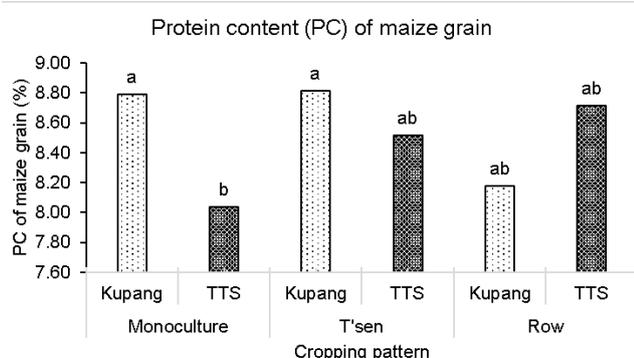


Figure 5. The protein content of maize affected by cropping patterns and varieties. The same letters in each chart were not significantly different based on Tukey's test ($p < 0.05$)

Table 4. Maize productivity, maize protein yield and total yield protein in various cropping patterns with maize varieties

Treatment	Maize productivity (t ha ⁻¹)	Maize protein yield (t ha ⁻¹)	Total protein yield (t ha ⁻¹)
Cropping pattern			
Monoculture	3.57 ^a	0.30 ^a	0.30 ^c
T'sen	3.27 ^{ab}	0.29 ^{ab}	0.64 ^a
Row	3.11 ^b	0.26 ^b	0.52 ^b
Variety			
Kupang	3.38 ^a	0.29 ^a	0.51 ^a
TTS	3.25 ^b	0.27 ^b	0.46 ^b

Note: The same letter in each column was not significantly different based on Tukey's test ($p > 0.05$)

Differences in productivity and protein content lead to differences in maize protein yield. Table 5 also shows that there is no interaction between cropping patterns and varieties on protein yield, but both treatments affect protein yield. Maize protein yield decreases in both *t'sen* and row cropping patterns, with *t'sen* cropping showing a non-significant decrease, resulting in a similar protein yield to monoculture. There is also no significant difference in protein yield between *t'sen* and row cropping patterns. The

Kupang variety has a higher protein yield compared to the TTS variety.

Table 4 also shows the total protein yield, which represents the overall protein content from all the plant components in each planting pattern. The interaction of cropping patterns and varieties did not affect the total protein yield, but there was an influence from each cropping pattern and variety. Both intercropping patterns, *t'sen* and row, led to a significant increase in total protein yield compared to monoculture. Notably, the *t'sen* cropping pattern resulted in a significantly higher increase in total protein yield than the row cropping pattern. Additionally, planting with the Kupang variety resulted in a higher total protein yield compared to the TTS variety. The enhanced total protein yield in the *t'sen* and row cropping patterns, compared to monoculture, occurred due to the production of cowpeas and pumpkins with their protein content in both cropping patterns (Table 5).

Table 5 also show the productivity, protein content, and protein yield of cowpea and pumpkin tends to differ if planted with the two intercropping patterns and if planted with each maize variety. The productivity of cowpeas and pumpkins in the row cropping pattern was respectively 7.2 and 4.3% higher than in the *t'sen* cropping pattern and 7.2 and 6.2% higher when planted with the TTS variety compared to the Kupang variety. At the same time, Table 5 shows that the protein content of cowpea and pumpkin is respectively 2.0 and 53.8% in the *t'sen* cropping pattern compared to the row cropping pattern, and 1.5 and 36.1% higher when planted with the Kupang variety than the TTS variety.

The condition of productivity and protein content of cowpeas and pumpkin causes differences in protein yield between the two intercropping patterns compared to monoculture and between the varieties used. Table 5 shows that the cowpea protein yield in the row cropping pattern and in planting with the TTS variety was 6.7% higher respectively compared to the *t'sen* cropping pattern and planting with the Kupang variety. Meanwhile, the protein yield of yellow pumpkin was 50.0% higher in the *t'sen* cropping pattern compared to the row cropping pattern, and when planted with the Kupang variety it was 33.3% higher than when planted with the TTS variety.

Correlation between observed variables and maize yields

The correlation analysis between the observed variables and maize productivity is presented in Table 6. Positive or negative correlation coefficient values indicate synergy or antagonism between variables, while large correlation coefficient values indicate the strength of the relationship. Table 5 shows a non-significant positive correlation with a very weak to weak closeness between chlorophyll a, b, total levels, and the chlorophyll a to b ratio with N nutrient uptake. Similarly, a non-significant positive correlation exists with a very weak or weak closeness between stomatal opening width and K nutrient uptake.

In addition, productivity and physiological activity exhibit different correlations. Productivity has a positive correlation with almost all observed variables, except that soil moisture content, N uptake, and the chlorophyll a/b ratio have a negative correlation. P uptake is positively and significantly correlated with a very strong closeness relationship. In comparison, K uptake, stomatal opening width, stomatal density, and transpiration rate are not significantly positively correlated with strong and moderate closeness. On the other hand, soil moisture content shows a negative correlation with a strong closeness, N uptake with a medium closeness, chlorophyll a to b ratio with a weak

closeness, photosynthesis rate, and seed protein content with a very weak closeness, and total chlorophyll with a weak closeness.

Discussion

The visual arrangement of maize, cowpeas, and pumpkin in *t'sen* and row cropping patterns creates a canopy with a distinct geometric structure divided into three strata. The top strata are occupied by corn due to its tall morphology, the middle strata are occupied by cowpeas, which grow climbing on maize stalks, and the lower strata are occupied by yellow pumpkin, which grows along the ground. These findings are consistent with previous research that has also observed the same geometric structure of the three plants in the *t'sen* cropping pattern (Dimu-Heo et al. 2022). Furthermore, the presence of cowpeas and pumpkins results in higher plant population densities in both intercropping patterns, leading to increased competition for growth resources, both in and above the soil, compared to corn monoculture. These conditions create dynamic relationships between plants and their environment, influencing physiological activities and productivity.

Table 5. Productivity, protein content and yield protein of cowpea and pumpkin fruit in various cropping patterns with maize varieties

Treatment	Cowpea			Yellow pumpkin		
	Productivity (t ha ⁻¹)	Protein content (%)	Yield protein (t ha ⁻¹)	Productivity (t ha ⁻¹)	Protein content (%)	Yield protein (t ha ⁻¹)
Cropping pattern						
<i>T'sen</i>	0.69	21.88	0.15	25.40	0.80	0.20
Row	0.74	21.45	0.16	26.49	0.37	0.10
Variety						
Kupang	0.69	21.83	0.15	25.16	0.72	0.18
TTS	0.74	21.51	0.16	26.73	0.46	0.12

Note: The data of cowpea and yellow pumpkin were not analyzed for variance

Table 6. Correlation coefficient between observed variables and maize yields

	NU	PU	KU	SW	SD	Ca	Cb	Ctot	RCab	TR	PR	PC	Productivity
SMC	0.86*	-0.81*	-0.67 ^{ns}	-0.48 ^{ns}	-0.26 ^{ns}	-0.15 ^{ns}	-0.18 ^{ns}	-0.23 ^{ns}	-0.02 ^{ns}	-0.16 ^{ns}	-0.43 ^{ns}	0.21 ^{ns}	-0.77 ^{ns}
NU	1.00	-0.56 ^{ns}	-0.22 ^{ns}	-0.35 ^{ns}	-0.40 ^{ns}	0.19 ^{ns}	0.20 ^{ns}	0.13 ^{ns}	0.09 ^{ns}	0.04 ^{ns}	-0.55 ^{ns}	-0.26 ^{ns}	-0.57 ^{ns}
PU		1.00	0.6 ^{ns}	0.32 ^{ns}	0.58 ^{ns}	0.34 ^{ns}	0.42 ^{ns}	0.43 ^{ns}	0.10 ^{ns}	0.70 ^{ns}	-0.16 ^{ns}	-0.30 ^{ns}	0.92**
KU			1.00	0.40 ^{ns}	-0.23 ^{ns}	0.59 ^{ns}	0.63 ^{ns}	0.63 ^{ns}	0.23 ^{ns}	0.20 ^{ns}	0.16 ^{ns}	-0.83*	0.55 ^{ns}
SW				1.00	-0.21 ^{ns}	-0.49 ^{ns}	-0.25 ^{ns}	-0.26 ^{ns}	-0.69 ^{ns}	-0.04 ^{ns}	0.45 ^{ns}	-0.04 ^{ns}	0.57 ^{ns}
SD					1.00	-0.01 ^{ns}	0.03 ^{ns}	0.05 ^{ns}	0.03 ^{ns}	0.71 ^{ns}	-0.46 ^{ns}	0.43 ^{ns}	0.53 ^{ns}
Ca						1.00	0.90**	0.90**	0.72**	0.27 ^{ns}	-0.31 ^{ns}	-0.74 ^{ns}	0.06 ^{ns}
Cb							1.00	0.99**	0.37 ^{ns}	0.33 ^{ns}	-0.26 ^{ns}	-0.65 ^{ns}	0.28 ^{ns}
Ctot								1.00	0.37 ^{ns}	0.29 ^{ns}	-0.20 ^{ns}	-0.61 ^{ns}	0.29 ^{ns}
RCab									1.00	0.16 ^{ns}	-0.35 ^{ns}	-0.54 ^{ns}	-0.29 ^{ns}
TR										1.00	-0.80 ^{ns}	-0.18 ^{ns}	0.60 ^{ns}
PR											1.00	0.16 ^{ns}	-0.01 ^{ns}
PC												1.00 ^{ns}	-0.10 ^{ns}
Yield													1.00

Note: ns: non-significant; **, *: significant correlation at the 0.01 and 0.05 probability level, respectively; SMC: soil moisture content; NU, PU, and KU: nitrogen, phosphorus, potassium uptake, respectively; SW, SD: stomatal width, and density, respectively; Ca, Cb, Ctot, and RCab: chlorophyll a, b, total and, ratio of chlorophyll a to b, respectively, TR, and PR: transpiration, and photosynthetic rate, respectively; PC: protein content; Yield: maize productivity

The initial condition of the land, as shown in Table 1, without any fertilization or irrigation relying solely on rainfall during the research period, describes the land as relatively marginal. In this research, intercropping in *t'sen* and row patterns positively modifies the growing environment, leading to the beneficial growth of the three plants involved in the intercropping. This is evidenced by the increased soil moisture levels and N nutrient uptake compared to monoculture. The increase in soil moisture levels in the *t'sen* and row cropping patterns is related to the cowpea canopy covering the middle of the plant and the spreading yellow pumpkin canopy covering the soil surface, reducing light interception. Reduced light interception keeps soil temperatures low, reducing evaporation and maintaining high soil moisture levels. The increase in moisture content in intercropping compared to monoculture has been discussed in previous reviews, indicating that high soil cover reduces evaporation, thereby increasing soil moisture (Ndiso et al. 2017; Ayele 2020; Silva et al. 2020; Ngapo et al. 2021; Perez-Hernandez et al. 2021).

Soil moisture levels generally affect the activity of plant stomata (Buckley 2019). Stomata opening allows the entry of CO₂ for photosynthesis and the loss of water through transpiration. Table 5 indicates that soil moisture content has a moderate relationship with stomatal opening width, with a negative value. This means that an increase in soil moisture levels causes a decrease in the width of corn stomata openings in both intercropping patterns. The research shows that in the *t'sen* cropping pattern, the decrease in the width of the stomata opening is smaller, so the width of the stomata opening is not significantly different from monoculture planting. On the contrary, in the row cropping pattern, the decrease in the width of the stomata opening is greater, resulting in a smaller width than in monoculture.

This research indicates that the stomatal width openings do not decrease in the *t'sen* cropping pattern, leading to a non-significant decrease in the rates of transpiration and photosynthesis compared to monoculture. On the other hand, the row cropping pattern leads to a decrease in transpiration rate, especially in the TTS variety, and a decrease in photosynthesis rate, especially in the Kupang variety, compared to monoculture. The decrease in transpiration rate in the row cropping pattern is considered due to the narrower width of stomatal openings compared to monoculture and *t'sen* cropping patterns. The decrease in photosynthesis in the Kupang variety is thought to be due to the lower stomatal density compared to the TTS variety (Table 3). These findings are consistent with Brownlee (2018) and Buckley (2019), who suggested that stomatal activity influences plant transpiration rates and photosynthesis.

According to Novak and Vidovicy (2003), water movement from transpiration affects nutrient absorption, as water carries dissolved nutrients. With a transpiration rate similar to monoculture, nutrient uptake, particularly of P and K, but not N, in *t'sen* and row cropping patterns is not significantly different. In denser plant populations, intercropping with *t'sen* and row cropping patterns does not

lead to differences in P and K absorption in maize plants. This is likely due to reduced nutrient competition from root zone depth variations among the three plants. Maize plants with a fibrous and shallower root system absorb nutrients from the top layer of soil, while cowpea and pumpkin plants with tap roots absorb nutrients from deeper soil layers. This aligns with Ndiso et al. (2017), who found that plants with deeper roots exploit more moisture and nutrients in deeper soil layers. A similar level of K uptake is believed to result in no difference in stomatal opening width, as the opening of stomata is also regulated by the presence of K⁺ ions (Brownlee 2018; Bertolino et al. 2019; Lawson and Violet-Chabrand 2019; Driesen et al. 2020).

The increase in N nutrient uptake in the two intercropping patterns compared to monoculture is attributed to N fixation by cowpeas, which can enhance the N content in the soil. This is consistent with previous findings that legume plants such as beans can increase the availability of N nutrients in the soil due to their fixation ability (Zhang et al. 2014; Kermah et al. 2018; Fan et al. 2020; Perez-Hernandez et al. 2021). However, we observed that the increase in N uptake in the *t'sen* cropping pattern did not lead to any differences from monoculture. In contrast, N uptake was significantly higher in the row cropping pattern than in monoculture. This difference may be attributed to the proximity of the root zones of the maize, cowpeas, and pumpkin in the *t'sen* cropping pattern, as they are planted in one hole. In contrast, the row cropping pattern allows maize plants to access more N nutrients due to wider spacing with other plants. This finding is consistent with Raza et al. (2019) and Fan et al. (2022), who found that narrower root zones due to closer planting distances increase competition between species in nutrient uptake.

The research shows that cropping patterns and varieties do not affect the content of chlorophyll a, b, and total chlorophyll in maize leaves. This indicated that the increased N nutrient uptake in intercropping with the *t'sen* and row patterns had not differentiated maize leaves' chlorophyll content compared to monoculture. The correlation analysis reveals a weak relationship between N uptake, chlorophyll content, and chlorophyll ratio. This is suspected the chlorophyll formation is influenced not only by N nutrients but also by other nutrient balance uptake (Ahmed et al. 2021; Fan et al. 2021) and possibly also by the use of N for protein synthesis and transfer to other parts of the plant (Ahmad et al. 2022). The same trend is also shown in the total chlorophyll content and chlorophyll a to b ratio in all cropping patterns, which are not significantly different. Generally, shaded leaves have a higher chlorophyll a to b ratio than unshaded leaves (Su et al. 2014; Liu and Zhang 2017; Fan et al. 2018; Fornari et al. 2020). This condition indicates that the shade influence on climbing cowpea canopy on maize stalks in the *t'sen* and row intercropping patterns does not cause an increase in chlorophyll b or a decrease in the chlorophyll a to b ratio compared to monoculture.

The dynamics of physiological activities, such as stomatal activity, chlorophyll content, transpiration rate, and nutrient uptake, result in different responses in photosynthesis rates,

ultimately affecting the productivity of both maize varieties in each cropping pattern. This study revealed that the same photosynthesis rate in the *t'sen* cropping pattern was not different from monoculture; the productivity of the two maize varieties was also not different from monoculture. In contrast, the photosynthesis rate in the row cropping pattern, especially in the TTS variety, was not different from monoculture, leading to a decrease in productivity, which was lower than monoculture. On the other hand, the Kupang variety, which had a lower photosynthesis rate, exhibited the same productivity as monoculture (Figure 4.B and Table 4). This finding is consistent with Faralli and Lawson (2019), who stated that photosynthesis is the main determinant of plant productivity, and any increase in photosynthesis has the potential to increase yields, depending on genetic variation in photosynthesis. The decrease in productivity among TTS varieties is believed to be due to a balanced distribution of photosynthate between generative and vegetative organs, the ability to transfer stored photosynthate from vegetative organs to grain during the grain-filling period, and the seed storage capacity. This has been suggested by various studies, including Zelitch (1982), Seebauer et al. (2010), da Silva et al. (2018), Hisse et al. (2019), Zhao et al. (2019), and Ren et al. (2022). Similarly, Sehgal et al. (2018) and Zhao et al. (2019) stated that the measured photosynthesis rate may better describe the instantaneous photosynthesis rate because the measurements are carried out at certain times and conditions, while the photosynthesis process is dynamic throughout plants growth and responds differently to changes in environmental conditions for each variety. Therefore, the results showing a high photosynthesis rate will not always lead to an increase in grain yield.

Additionally, the research indicates an impact on maize productivity; intercropping patterns influence the protein content quality, particularly in the TTS variety (Figure 5) than monoculture. In monoculture planting, the TTS variety has lower grain protein content than the Kupang variety. Still, with *t'sen* and row cropping patterns, the protein content of the TTS variety increases and becomes similar to the Kupang variety. Meanwhile, the protein content between the *t'sen* and row cropping patterns did not differ. The difference in protein content in our study is believed to be related to N uptake, which was also not different between the Kupang and TTS varieties in the two cropping patterns. This confirms previous findings that protein content is influenced by N uptake as one of the main ingredients for protein formation (Seebauer et al. 2010; Zhang et al. 2020).

Therefore, from an intercropping perspective, the differences in physiological activities and yields of maize in *t'sen* and row cropping patterns are also due to the division and distribution of growth resources for the yield needs of cowpeas and pumpkins (Table 5). The research showed that planting cowpeas and pumpkin in the *t'sen* cropping pattern results in higher productivity, while using the row cropping pattern results in higher total protein yield compared to monoculture, as shown in Tabel 4. The presence of production, protein content, and total protein yield in *t'sen* and row cropping patterns highlights the advantages of these intercropping patterns in terms of quantity and

diversity. These results are consistent with previous findings that intercropping produces higher total crop yields, both production and protein content, than monoculture (Pierre et al. 2018; Elsaid et al. 2019; Bo et al. 2022).

The research indicated that planting maize with the *t'sen* cropping pattern leads to present higher soil moisture levels. However, it does not affect differences in the physiological response of the two maize varieties across various observed variables such as NPK nutrient uptake, stomata density, chlorophyll content (a, b, and total), chlorophyll a to b ratio, transpiration rate, and photosynthesis rate; therefore, the productivity is not significantly different from monoculture planting. Similar conditions were observed in the row cropping pattern, except for an increase in N uptake and a decrease in the transpiration rate and photosynthesis rate in the Kupang variety, leading to decreased plant productivity. Both intercropping patterns also increased seed protein content, particularly in the TTS variety, comparable to the Kupang variety; both intercropping patterns increased productivity and protein content in cowpea grains and pumpkin fruit compared to monoculture. The research results indicate that the *t'sen* cropping pattern is more profitable than monoculture and row planting, making it viable option for planting on marginal land with minimal production inputs. This includes limited fertilizer and irrigation due to unpredictable rainfall. Further research on the *t'sen* cropping pattern is needed due to the limited information on its development.

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