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We are now here with our Vol. 4 No. 2 issue, thank you very much to our editors, writers and referees who contributed to this issue.

Best regards

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Research Article

Winter hardiness in Lentil (*Lens Culinaris* Medik)

Selin Gunduz[1], Abdulkadir Aydogan[2], Havva Vildan Kilinc[3], Elif Atasayar[4] and Ersin Kavlak[5]

Field Crops Central Research Institute, Republic of Turkey Ministry of Agriculture and Forestry, Ankara, Turkiye

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Abstract

Climate change affects lentil (Lens culinaris Medik.) cultivation, particularly through extreme winter weather, highlighting the need to assess winter hardiness. This study conducted on of four advanced lentil lines and three controls under winter sowing conditions over three successive growth periods from 2021 to 2023 at the Ikızce Research Application Farm of Field Crops Central Research Institute, Turkey. The analysis aimed to determine the variability in yield and winter resilience of red lentil lines. Results releaveled negligible winter damage, with plant heights ranging from 24 to 30 cm, first pod heights from 23 to 33 cm, and 50% flowering occurring on average of 196 days. Principal Component Analysis (PCA) revealed that 75% of observed variability between genotypes are attributed to genetic factors, while 25% can be ascribed to external influences. Notably, lines AKM 1089 and AKM 1087 have been detected as superior, providing a foundation for future lentil breeding studies to develop climate-resilient varieties with genotypic superiority.

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Introduction

Climate change is causing long-term alterations to global climatic patterns, including changes in temperature, precipitation, and wind direction (UN, 2022). It is important to take action to mitigate the effects of climate change. Developing new varieties of lentil is crucial in the face of climate change that threatens global food security. Millions of people worldwide rely heavily on grain legumes as a source of protein in their diets, making them a vital component in maintaining global food security (Jha et al., 2022).

Among grain legumes, lentil (*Lens culinaris* Medik.) is an annual grain legume that has been domesticated as the first crop since 7000 BC (Erskine et al., 2016) in the Fertile Crescent (Ladizinsky, 1979). It is the third most significant cool-season grain legume after chickpeas and peas worldwide (Sehgal et al., 2021). More than 40 countries cultivate lentils, and the top producers are the US, Canada, India, Australia, and Türkiye (FAO, 2021). According to Kaale et al., (2023),

1 Corresponding Author: MSc., Field Crops Central Research Institute, Republic of Turkey Ministry of Agriculture and Forestry, Ankara, Turkiye. E-mail: selin.egilmez@tarimorman.gov.tr ORCID: 0000-0003-4762-4876
2 Dr, Field Crops Central Research Institute, Republic of Turkey Ministry of Agriculture and Forestry, Ankara, Turkiye. E-mail: abdulkadir.aydogan@tarimorman.gov.tr ORCID : 0000-0002-9747-4004
3 MSc., Field Crops Central Research Institute, Republic of Turkey Ministry of Agriculture and Forestry, Ankara, Turkiye. E-mail: havvavildan.aydin@tarimorman.gov.tr ORCID : 0000-0002-7812-4041
4 Field Crops Central Research Institute, Republic of Turkey Ministry of Agriculture and Forestry, Ankara, Turkiye. E-mail: elifyilmaz@tarimorman.gov.tr ORCID : 0000-0001-8155-5152
5MSc., Field Crops Central Research Institute, Republic of Turkey Ministry of Agriculture and Forestry, Ankara, Turkiye. E-mail: ersin.kavlak@tarimorman.gov.tr ORCID: 0009-0005-1108-1146

the total production amount of lentil climbed by 107% globally in the two decades (2001–2020), from 3.15 to 6.54 million tonnes. Specifically, in Türkiye, where the experiment was carried out, lentil is produced as red and green cultivars, winter and summer, respectively. 98% of it is cultivated for winter in the South-eastern Anatolia and Central Anatolia Regions (Aydoğan et al., 2008).

Lentil is a nutrient-dense plant. It has a high content of protein 22% to 35%, minerals, vitamin content and complex carbohydrates with high energy levels (Dhull et al., 2020). In this respect, its grains are suitable for human consumption and its dry straw is suitable for use as animal feed. In addition to its nutritional properties, lentils fix atmospheric nitrogen in root nodules through a symbiotic relationship with Rhizobium and contribute to increased soil fertility and health (Teng et al., 2015).

Lentil, a cool-season legume that can grow in various soil types and requires an average of 300-400 mm of annual rainfall (Asakereh et al., 2010). However, the production of lentil is limited by several biotic and abiotic factors, including drought, salt, excessive temperature, and mineral shortage (Sehgal et al., 2021). To ensure the sustainability of lentil production, it is crucial to develop new varieties that are resilient to these challenges. Lentil growing in highland regions is challenging due to its vulnerability to freezing temperatures (Kahraman et al., 2004). Nightly frost can decrease seed yields by 15.5 kg/ha per day, and cold can cause a decrease in large leaf and stem mass and yield (Erskine et al., 1993). Despite this, winter-sown lentils can produce 50% to 100% more than spring-sown lentils (Sakar et al., 1998).

To develop climate-resilient and high yielding cultivars, it is crucial to comprehend the relationships that exist between yield and the other plant characteristics (Karadavut, 2009). Although several studies have been carried out to analyse yield and yield components of lentil. This research is essential since analyses recent data on winter lentil plantings in the central Anatolia region. It provides the infrastructure for future lentil breeding studies in the region to see the effects of external factors on the plant yield.

Problem of the study

Assessment of Winter Hardiness in Lentil Lines: Investigate the resilience of various lentil genotypes to winter conditions, focusing on their ability to withstand cold temperatures and other stress factors during the winter season.

Evaluation of Genotype and Environmental Influences on Lentil Yield: Analyse the impact of both genetic factors and external affects on the yield and yield components of different lentil genotypes, aiming to identify key determinants of productivity.

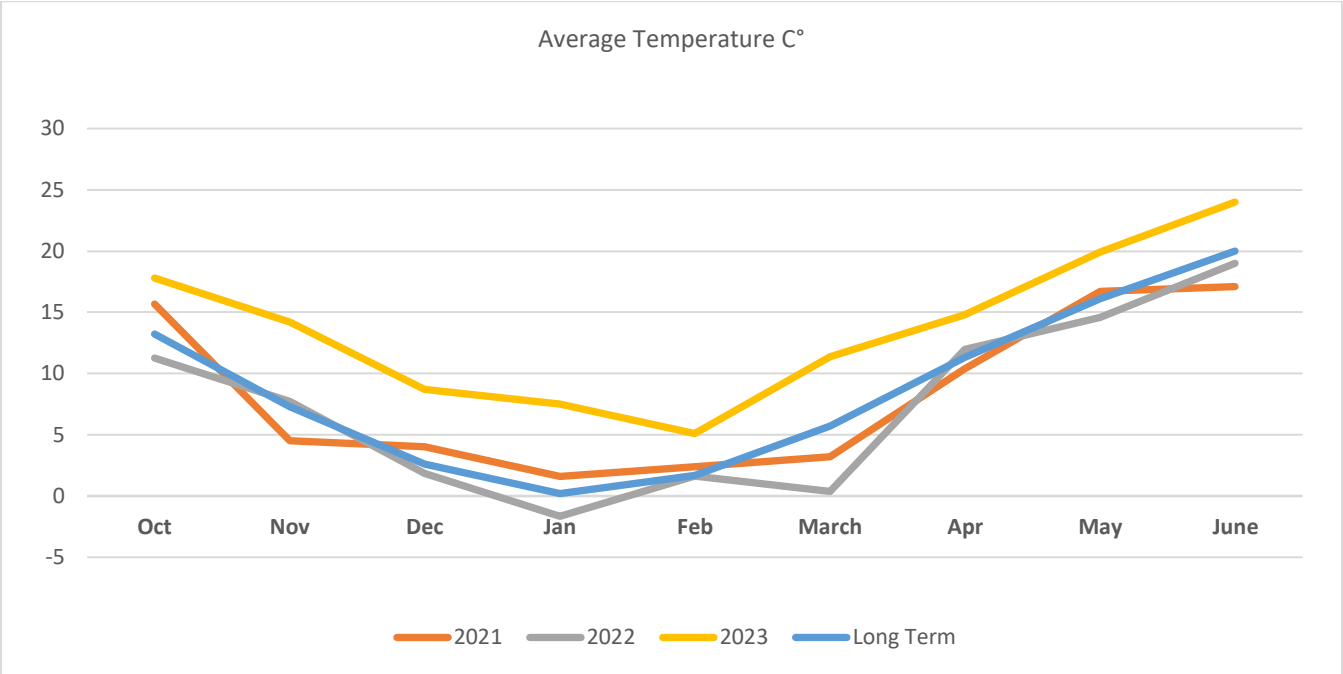
Identification of Superior Lentil Lines for Winter Sowing: Detect lentil lines that demonstrate exceptional performance in winter conditions.

Material and Methodology

The study was conducted as a field trial from 2021 to 2023 as winter sowing (October to June). Four red lentil genotypes 'AKM 1021, AKM 1077, AKM 1087, AKM 1089', and three checks 'Şakar, Çiftçi, Fırat'. The trial was established in the Randomized Blocks trial design, with three replications, on plots of 5.5 m^2, with 350 seeds per m^2, at the Field Crops Central Research Institute İkizce Research Institute, Ankara, Türkiye under rainfed conditions. There was no fertiliser applied during the trial.

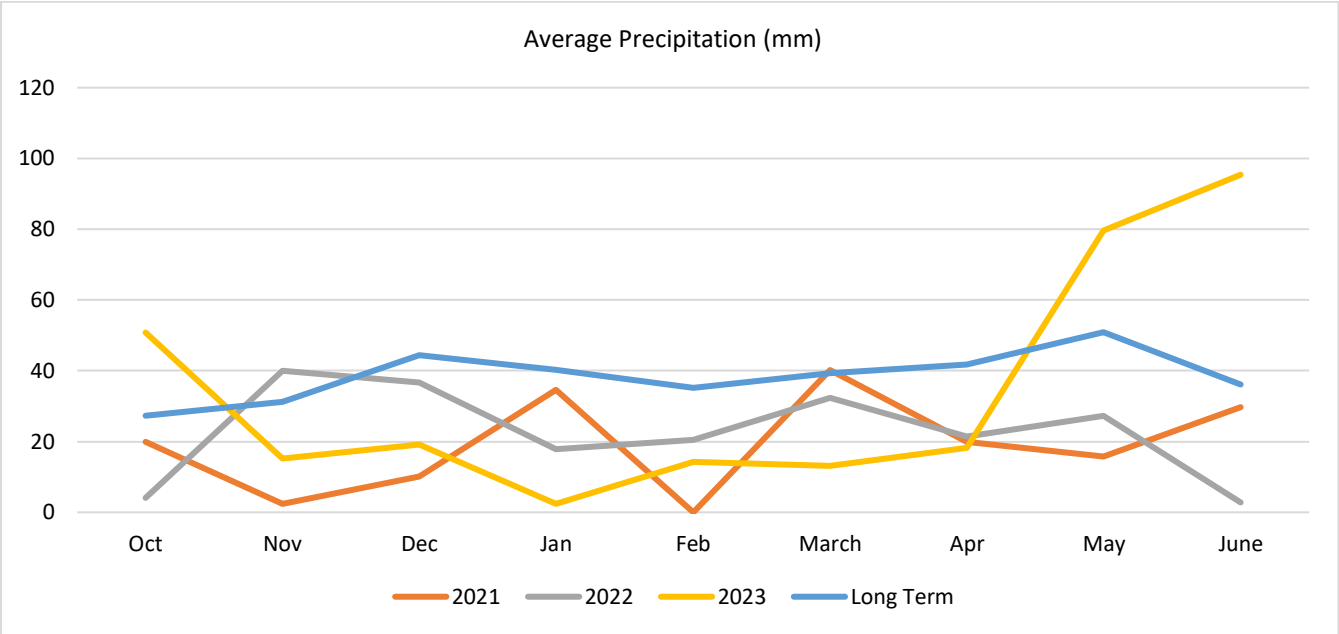
The soil type of the experiment field is in the brown soil group, clayey, loamy soil structure. The pH level of the soil is 7.8, and its structure leans slightly towards alkalinity. Soil CaCO3 content is 33.3% (Karakurt, 2012).

Figure 1-2 shows the variation in average precipitation (mm) and average temperature (°C) during the experimental period. Temperature and precipitation were increased over the years during the growing periods. The lowest temperature was observed in the second year of the trial with -1.7 °C, while the highest temperature was observed in June last year with 24 °C in total, precipitation has increased over the years, including 173 mm, 202.6 mm, and 308.3 mm.



Source: General Directorate of Meteorology

Figure 1. The average temperature of the experiment period



Source: General Directorate of Meteorology

Figure 2. The average rainfall amount during the experiment period

Morphological observations were made during each year of the trial period according to IBPGR, (1985). The number of days for 50% flowering, plant height, first pod height, and winter damage were observed, respectively, starting from the germination date. In particular, an assessment of winter damage (Singh et al., 1989) was carried out in April using a scale of 1 to 9 (1 is resistant and 9 is sensitive). Evaluations include post-harvest thousand-seed weight (g) and calculation of yield (kg/da).

For evaluating the obtained data, statistical analyses were carried out with the JMP15 statistical software. Groupings were made by applying variance analysis and LSD test, and the standard error of the mean (S.E.), and coefficients of variation (C.V.) (Steel et al., 1997) were tested to test the importance of the differences between the means.

Results and Discussion

The effects of winter damage on lentil lines and varieties that were acquired concurrently with climate data are shown in Table 1. Over time, lines and varieties' ability to withstand cold damage during the growing season has changed, both

about one another and in individual analyses. The plant development period, the blooming period, and the period immediately preceding flowering were used to evaluate the extent of winter damage. Then their averages were calculated. Following Akçin's findings (1988), lentils have demonstrated resilience to short periods of low temperature, with the capacity to endure temperatures as low as -12 °C. The extent of winter damage was quantified on a scale ranging from 1 to 5, as detailed in Table 1. Notably, no severe damage was detected during the study, indicating a certain level of winter hardiness among the evaluated lentil lines and varieties.

Table 1. Winter hardness observations averages

2021	Score	2022	Score	2023	Score
AKM 1021	2	AKM 1021	3	AKM 1021	2
AKM 1087	3	AKM 1087	2	AKM 1087	1
AKM 1089	2	AKM 1089	3	AKM 1089	2
AKM 1077	3	AKM 1077	2	AKM 1077	1
Şakar	3	Şakar	5	Şakar	2
Fırat 87	3	Fırat 87	2	Fırat 87	2
Çiftçi	3	Çiftçi	3	Çiftçi	2

According to Kahraman et al., 2004 it is important to ascertain relationships between various plant characteristics and winter hardiness. Thus, the study considered the agronomic features of the plant as well as yield and yield components.

Yield values of lines and varieties have changed over the years (Table 2). It was found statistically significant at 5%. The statistical analysis of yield values throughout the trial period revealed that the genotypes consistently demonstrated values surpassing the controls. According to the three-year average, the highest efficiency average was observed in AKM 1087 and AKM 1089. When compared based on lines and varieties, AKM 1087 received the highest value in the second year of the trial with 224 kg/da; when the varieties were compared, the Çiftçi variety showed the highest value with 188 kg/da in the same year. Notably, these results surpass the findings of a previous study by Aydoğan et al. (2008), where the highest yield reported was 176.2 kg/da.

Despite the Şakar variety exhibiting the highest winter damage with a score of 5 points in the second year of the experiment, no significant differences were observed in yield values over the years. It is worth noting that harsh winter conditions lead to yield losses, as stated by Bélanger et al. (2006).

In the year 2021, the Fırat87 variety displayed the lowest yield, and in 2022, the Şakar variety showed the second lowest yield at 116 kg/da and 132 kg/da, respectively. It is interesting to highlight that, contrary to the study conducted by Çölkesen et al. (2014), where the Fırat 87 variety exhibited yields ranging from 180.50 to 243.68 kg/ha with an average of 212.09 kg/ha during the 2011-12 seasons, the material tested under current conditions achieved the highest yield of 166 kg/ha in 2023. This suggests an increase in the yield value of the cultivar over the years, displaying its adaptability or potential improvement under the specific conditions of the experiment.

Numerous studies, including Mekonnen et al. (2014), have consistently reported variations among lentil genotypes in terms of per-plant yield. Additionally, Toklu et al. (2015) have noted substantial phenotypic diversity within lentil genotypes. These findings underscore the importance of understanding and harnessing genetic and phenotypic variability in lentil populations, providing valuable insights for breeding programs and cultivation practices aimed at optimizing yield and overall crop performance.

Table 2. Yield evaluations (kg/da)

		Year			
Genotypes	**2021**	**2022**	**2023**	**Least Sq Mean**	**Range**
AKM 1087	152	224	206	194	A
AKM 1089	147	208	206	187	A
AKM 1077	134	187	219	180	A
ÇİFTÇİ	160	188	168	172	AB
AKM 1021	144	208	159	171	AB
FIRAT 87	116	153	166	145	BC
ŞAKAR	137	132	143	137	C
Source	**DF**	**Sum of Squares**	**F Ratio**		
Year	2	24812,794	13,5594**		
GEN	6	24274,159	4,4217**		
GEN*Year	12	13896,984	1,2657 **ns**		
Rep[Year]	6	4065,81	0,7406 **ns**		
Error	36	32938,857	914,97		
C. Total	62	99988,603			
%CV	17,85990148				
LSD	28,91914374				

The thousand-seed weight was found to be statistically significant at the 1% level. Over the years, there has been a noticeable downward trend in the values of lines and genotypes. The average thousand-seed weight for lines and varieties across years is 39 g, as detailed in Table 3, which provides comprehensive statistics and reference ranges.

Lines and varieties exceeding the trial averages for thousand-seed weight across years are highlighted in the table. The recorded values range from the lowest of 34 g in Fırsat87 to the highest of 49 g in Şakar. Notably, among the lines, AKM 1077 stands out with a superior value of 43 grams.

Comparing the advanced lentil lines to the check varieties reveals a greater phenotypic diversity, a finding consistent with Toklu et al. (2017). Additionally, Tyagi et al. (2011) reported significant differences in 1000-seeds weight between lines and checks. These observations underscore the importance of considering and leveraging such diversity for optimizing seed weight in lentil breeding programs.

Table 3. Statistical analysis of 1000-seeds weight

		Year			
Genotypes	**2021**	**2022**	**2023**	**Least Sq Mean**	**Range**
ŞAKAR	49	41	40	43	A
AKM 1077	43	37	37	39	B
ÇİFTÇİ	40	35	40	38	B
AKM 1087	39	38	37	38	B
FIRAT 87	43	35	34	38	BC
AKM 1021	39	35	38	37	BC
AKM 1089	38	35	36	36	C
Source	**DF**	**Sum of Squares**	**F Ratio**		
GEN	6	250,46444	11,6144*		
Rep[Year]	6	46,14381	2,1398		
Year	2	295,93556	41,169		
GEN*Year	12	168,56889	3,9084		
Error	36	129,38952	3,5942		
C. Total	62	890,50222			
%CV	4,9228002				
LSD	1,812504033				

Table 4 presents the morphological data evaluation for the lentil genotypes. Notably, plant height, a crucial factor for harvesting and pod binding, exhibited variations across the genotypes. Fırat87 displayed the highest plant height, reaching 33 cm in the final year of the experiment, with the peak values observed in the third year of the trial. Control varieties exhibited the shortest and longest values for plant height, with Fırat87 recording the longest value in 2023 (33 cm) and Çiftçi registering the shortest in 2021 (23.7 cm).

In terms of lines, AKM 1089 and 1077 achieved the longest values at 30 cm and 31 cm, respectively, in 2023. Additionally, the first pod height exhibited an average ranging from 7 cm to 16 cm among the lines. The three-year average for the 50% flowering time was reported as 196 days, a trait known to significantly influence lentil yield. Comparing these findings to a lentil breeding study conducted at the same location in 2001 and 2003, where flowering dates changed from 197 to 261 days, underscores the dynamic nature of flowering traits over time (Kahriman et al., 2015). These observations emphasize the importance of understanding and monitoring morphological traits for informed lentil breeding strategies.

Table 4. Morphological observations

	2021			2022			2023		
Genotypes	**%50 f.d**	**p.h.**	**f.p.h**	**% 50 f.d**	**p.h.**	**f.p.h**	**% 50 f.d**	**p.h.**	**f.p.h**
AKM1021	189	24,7	12	208	25	15	196	29	8
AKM1087	189	24,7	12	206	28	13	195	26	8
AKM1089	189	24,7	12	204	26	16	196	31	8
AKM1077	187	26,7	12	206	27	14	194	30	8
Şakar	188	26,3	13	204	25	10	191	31	9
Fırat 87	188	24,3	11	209	25	16	196	32	7
Çiftçi	189	23,7	12	208	26	15	195	28	7
Min	187	23,7	11	204	25	13	191	28	7
Max	189	26,7	13	209	28	16	196	32	9
Average	189	25	12	206	26	14	194	29	8

(%50 f.d.: 50% Flowering days, p.h.: Plant Height (cm), f.p.h: First pod height (cm))

Upon evaluating the stability of the genotypes was evaluated with respect to the yield and thousand-seed weight (Figure 3, 4), it was observed that AKM 1077 and AKM 1087 had high stability over the years, besides having high yield. However, while Şakar has the lowest stability in terms of yield value, it has the highest stability in terms of thousand-seed weight.

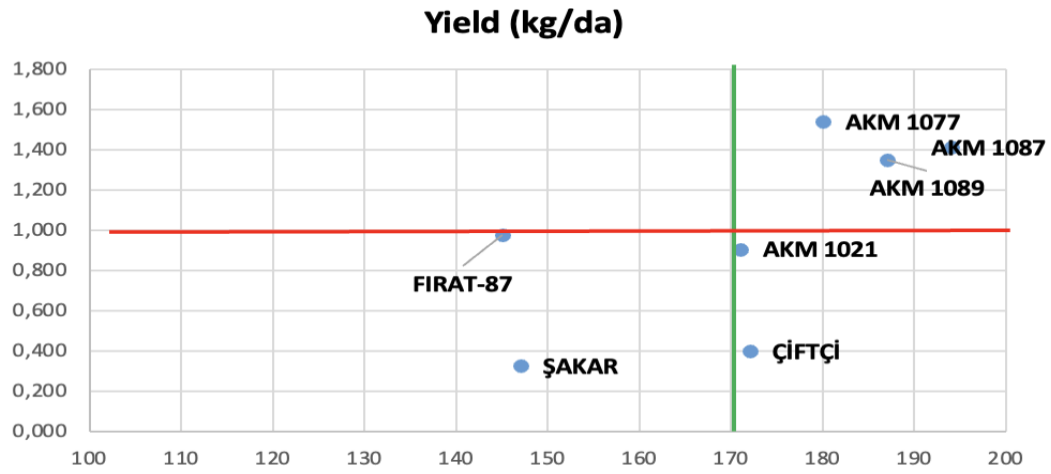


Figure 3. The stability analysis of yield value

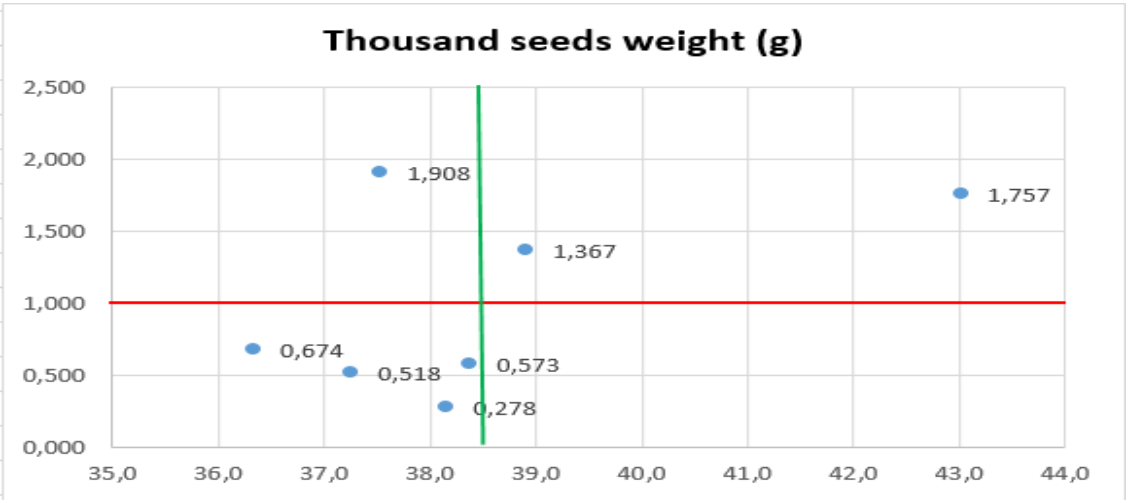
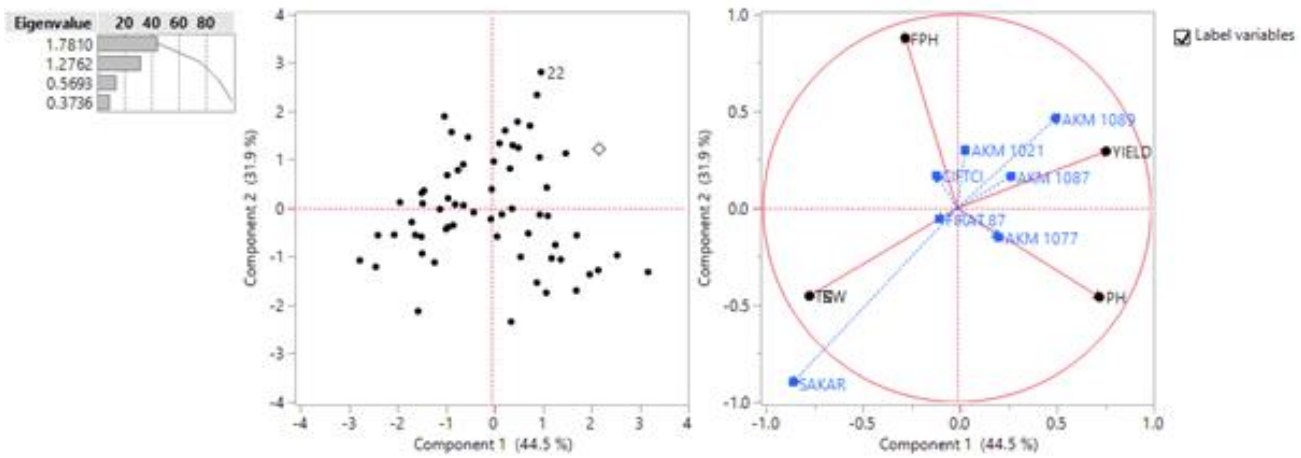


Figure 4. The stability analysis of thousand-seed weight

With the principal component analysis (Figure 5), it was shown that the proximity of the lines to each other and their superiority are specified in the figure. For plant breeding comprehension the basis of genotype × environment (GxE) interaction is vital (Subedi et al., 2021). According to the principal component analysis, results suggest that the majority (75%) of the variation observed in the dataset is attributed to differences between genotypes. The remaining 25% of the variation is attributed to external factors. These external factors could include environmental conditions, management practices, or any other non-genetic influences that contribute to the observed differences among the genotypes. It indicates that a significant portion of the observed differences is genetically determined, while a smaller proportion is influenced by external, non-genetic factors. The findings suggest that a substantial portion of the observed variation can be explained by genetic factors, reinforcing the importance of genotype-related differences in the context of the studied plant population.



(ph: plant height, tsw : thousand seed weight, fph: first pod height)

Figure 5. Principal component analysis

Conclusion

Climate change is exerting a significant influence on lentil yield and yield components, primarily due to irregular alterations in temperature and rainfall. A recent three-year study conducted under Ankara Haymana conditions, focusing on seven genotypes, yielded noteworthy findings. Observations on winter damage indicated some impact between lines from 1 to 5, although it was not particularly pronounced. The findings of the study suggest that a significant portion (75%) of the observed variation within the studied plant population can be attributed to genetic factors. This reinforces the pivotal role of genotype-related differences in shaping the traits and characteristics under investigation. The remaining 25% of the variation is influenced by external factors, highlighting the complex interplay between genetic and environmental elements.

Recommendations

Key outcomes of the study identified specific lines, namely AKM 1077 and AKM 1087. These lines demonstrated high yield potential, resilience against winter damage, substantial thousand-seed weight, and remarkable vegetative characteristics. This updated and comprehensive dataset contributes valuable insights for future lentil breeding studies, particularly in the context of addressing climate change challenges.

Data Availability

Researchers who want to access data for further analysis should use relevant data and documents; For any questions or requests regarding the dataset, please contact the corresponding author.

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Research Article

The process of producing Paedrew Giant seabass with the participation of farmers to register for geographical indication

Sataporn Deeying[1] and Tossapone Raumchimplee[2]

Department of Agricultural Technology, Faculty of Science and Technology, Rajabhat Rajanagarindra University, Chachoengsao, Thailand

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Abstract

The research aims to study and compile the production process of PaedRiew Giant Sea Bass with local farmers and analyze this process in preparation for Geographical Indication (GI) registration. The study focused on 29 groups of giant sea bass farmers in Bang Kluea Sub-district, Bang Pakong District, Chachoengsao Province. A participatory approach was employed to understand the production process, including ecological concepts and practical techniques for cultivating giant sea bass. Data were collected directly from the production areas, and the information was presented using visual flow diagrams, accompanied by detailed descriptions. The findings highlight that the giant sea bass farming group in Bang Kluea is characterized by advanced aquaculture techniques, expert farm management, superior fish breeds, and high-quality water. These factors enable year-round farming of giant sea bass, which typically reach 5-8 kilograms after 18-20 months, due to the unique salinity conditions of the Bang Pakong River. In contrast, other areas can only raise smaller white snapper (700-900 grams) within 4-5 months. The Chachoengsao group practices a comprehensive farming process, from breeding to growing fish to giant size, ensuring high survival and growth rates. The fish produced are known for their large size, firm and chewy texture, high nutritional value (rich in omega 3 and 6), and absence of fishy odor, thanks to a specialized bleeding technique called "Ike-jime," adapted from Japanese practices. This technique maintains the freshness and quality of the fish, resulting in white, clear flesh with a good texture. Additionally, the fish exhibit a rainbow-colored sheen due to a curing process that integrates fat into the flesh. The entire production process is divided into three main stages: the production of white snapper fry, raising the fish to market size, and growing them into giant sea bass. Throughout each stage, there is a strong emphasis on maintaining high standards of quality, which is crucial for meeting the criteria required for GI registration. This comprehensive approach not only enhances the product's market value but also positions PaedRiew Giant Sea Bass as a unique and high-quality product suitable for both local and international markets.

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Introduction

Giant seabass has key breeding areas in the provinces of Chachoengsao, Surat Thani, Nakhon Si Thammarat, Pattani, and Songkhla, which together account for 62.10% of the total farming area, 56.44% of the total number of farms, and 77.03% of the total aquaculture production (Department of Fisheries Policy and Strategic Development, Department

[1] Assistant Professor, Rajabhat Rajanagarindra University, Chachoengsao province, 24000, Thailand. E-mail: sataporn.deeying@gmail.com ORCID: 0009-0005-8216-4918
[2] Lecturer, Rajabhat Rajanagarindra University, Chachoengsao province, 24000, Thailand. E-mail: tossapone1971@gmail.com ORCID: 0009-0001-7687-827X

of Fisheries). Giant seabass is an economically significant fish that can be bred and raised in earthen ponds in both brackish and saltwater environments. Nearly 100% of the seabass sold in the market are farmed, with a typical market size known as "plate-size seabass" weighing approximately 700-900 grams. However, the giant seabass of Chachoengsao Province, known for its larger size of 5-8 kilograms, is particularly distinctive. This variety has firm, thick flesh, a delicious flavor, a fresh scent, high Omega content, and is free from any fishy odor. The product is available year-round, which sets it apart from seabass in other provinces.

Additionally, giant seabass in Chachoengsao Province is exclusively bred and raised locally from juvenile to adult stages. This uniqueness has driven the farmers to seek Geographical Indication (GI) registration for giant seabass as a provincial specialty product. GI registration would standardize the product, build consumer confidence, and highlight its unique characteristics, ultimately increasing farmers' income through the sale of a distinctive and standardized product in global markets.

The GI registration process requires comprehensive preparation, including geographical and biological information, production factors, and management processes. Farmers and stakeholders must be involved in every step to emphasize the importance of participation in preparing for GI registration. For giant seabass farming, this includes planning, pond preparation, broodstock selection, fry release, feeding, sorting, and harvesting. It is essential to support and validate these practices with scientific data to confirm the unique identity of Paet Rio giant seabass and prepare for its GI registration

Objectives

- To study and compile the context of the Paet Rio giant seabass production process in collaboration with farmers.
- To analyze the Paet Rio giant seabass production process in preparation for Geographical Indication (GI) registration.

Research Framework

The study of the participatory management process for the production of Paet Rio giant seabass by farmers will be conducted under the theories and concepts related to giant seabass production. This will be linked with the preparation of information for GI registration in Thailand, with an emphasis on participation. The study will collect comprehensive data related to 1) seabass fry production, 2) raising seabass to market size, and 3) raising seabass to full-grown giant size, with the aim of identifying the unique geographical indications of Paet Rio giant seabass. The conceptual framework is summarized as follows:

Giant sea bass farming is a unique area with distinctive farming characteristics, this led to farmers' demand to upgrade the giant seabass to become a provincial product by requesting registration as a Geographic Indication (GI) product to become a standard product, build confidence among consumers who are interested in products with special features, it is unique, which will lead to increased income for farmers from selling unique products and standard products that will lead to trade in the world market, which the application for GI registration must have a process for preparing all-around information, both geographic information and biological information of the area, production factors, production management processes to obtain comprehensive and important information in preparing for GI registration.

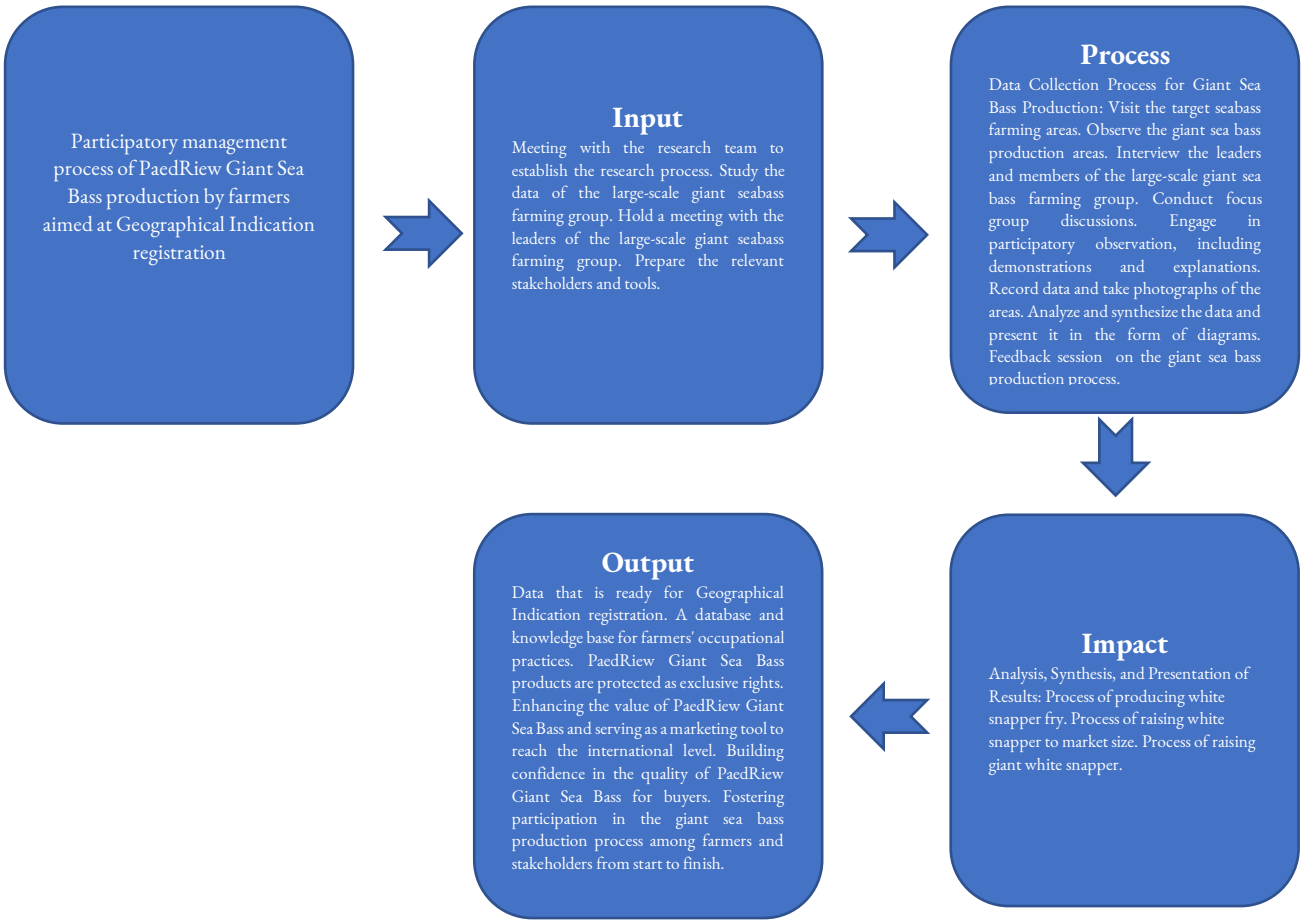

Figure 1. The participatory management process of PaedRiew Giant Sea Bass

Research Methodology

Population and Sample Group

The population consists of giant seabass farmers in Bang Pakong District, Chachoengsao Province.

The sample group includes a network of 29 large-scale giant seabass farmers in Bang Kluai Subdistrict, Bang Pakong District, Chachoengsao Province.

Research Instruments

The research instruments for this study include participatory spatial analysis of the giant seabass production process and the application of the Ecosystem Approach to Aquaculture (EAA) for giant seabass farming. Data collection tools include direct observation of production areas, photography of the production process, and the presentation of the data in the form of process flow diagrams, which serve as the primary medium for presenting the findings, along with descriptive explanations of each diagram.

Research Procedure

The participatory production management process for giant seabass farming in Paet Rio, aiming for Geographical Indication (GI) registration, involves the following steps:

Step 1. Preparation before studying the giant seabass production process.

Step 2. Data collection in the production areas of giant seabass.

Step 3. Data compilation, analysis, and presentation.

Step 4. Presentation and feedback of the giant seabass production process data.

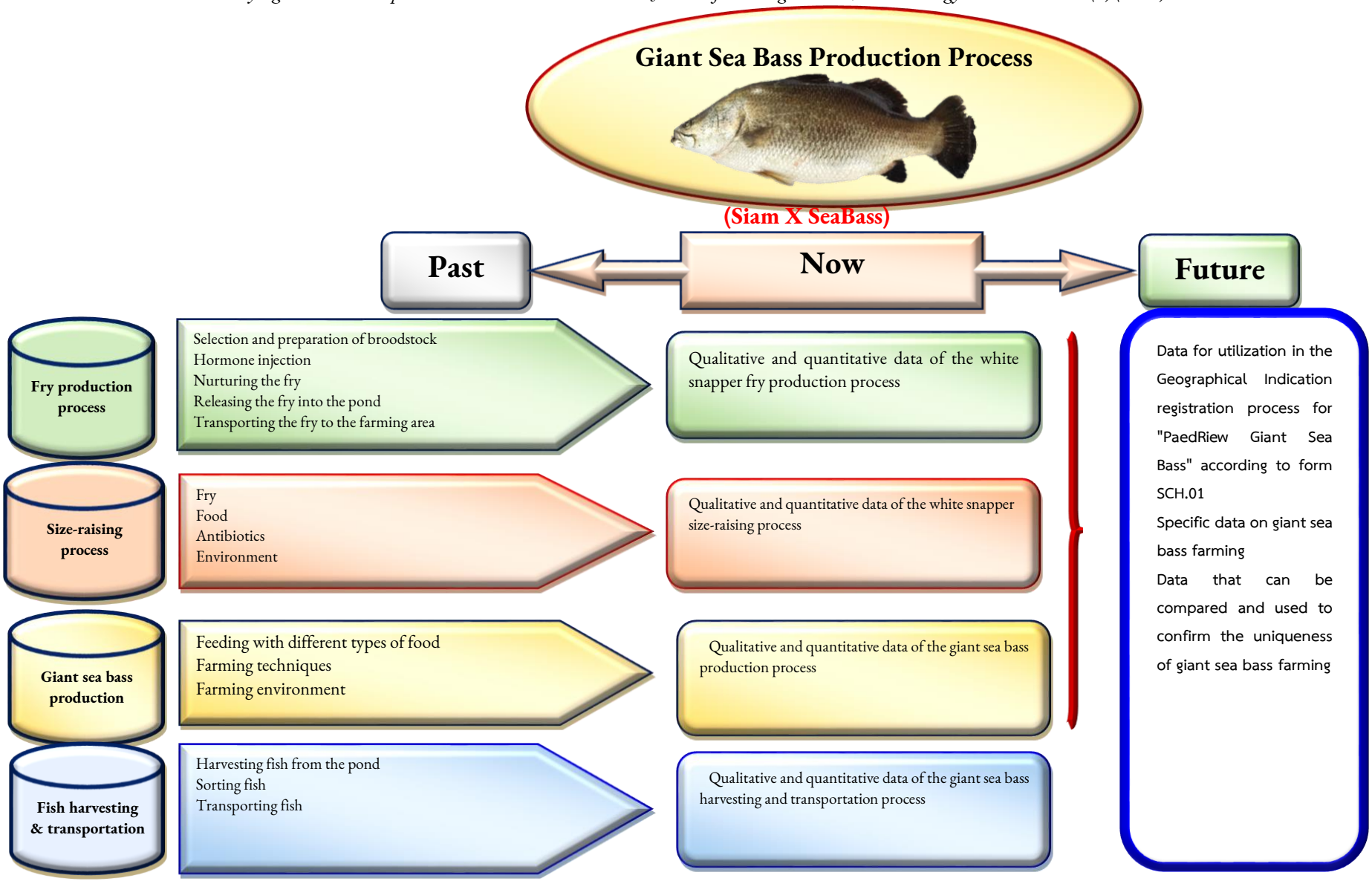

Figure 2. Research plan for the participatory production process of PaedRiew Giant Sea Bass by farmers aimed at Geographical Indication registration.

Research procedure

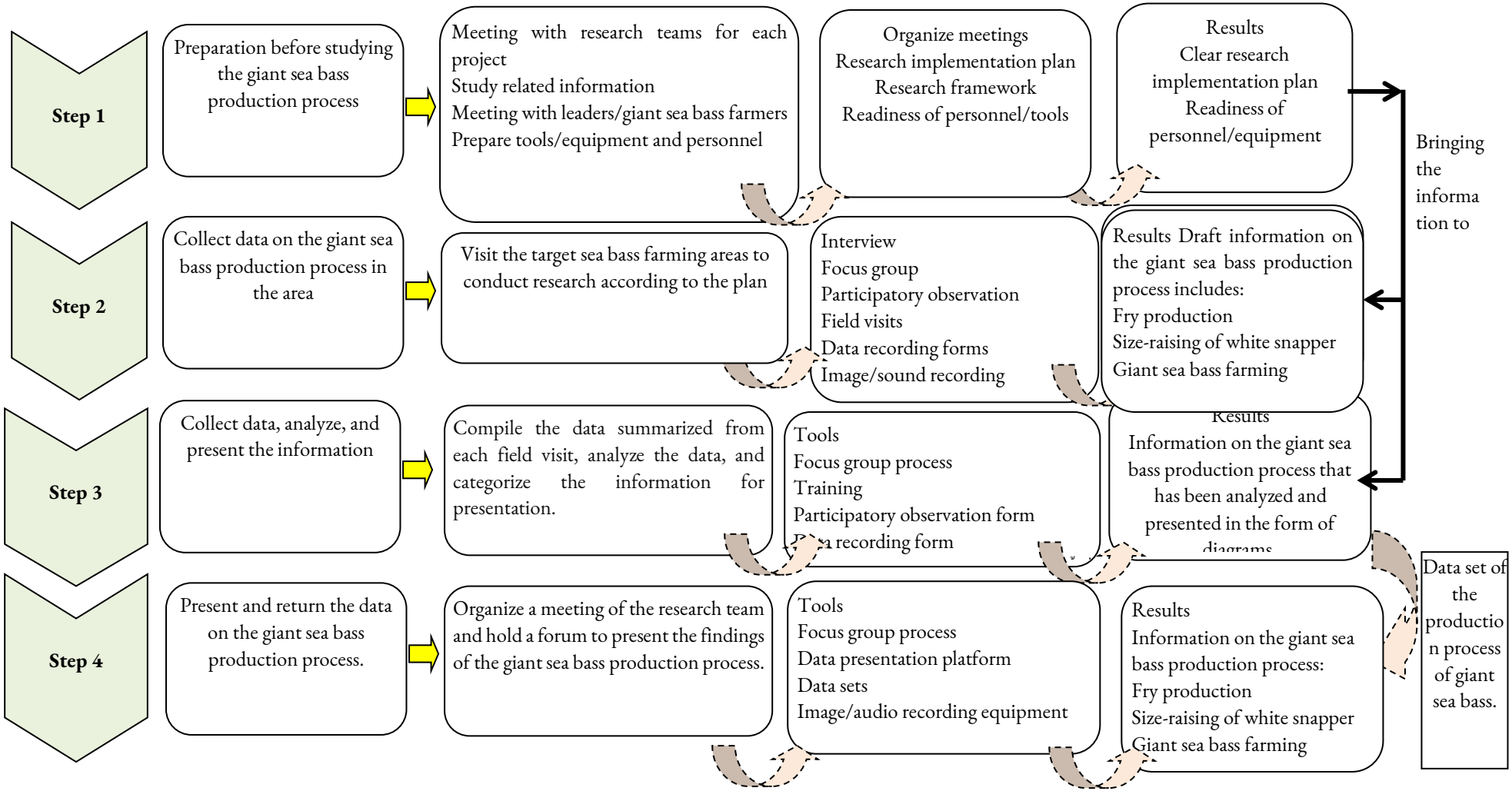

Figure 3. Research implementation steps

Research Findings

The giant seabass producer group in Bang Kluai Subdistrict, Bang Pakong District, Chachoengsao Province, is a large-scale enterprise consisting of 29 members. The group's strength lies in their advanced cultivation techniques, where the seabass farmers possess significant expertise, effective farm management, superior fish breeds, excellent water quality, and in-depth knowledge of rearing giant seabass. These key strengths provide them a competitive advantage over other regions that cannot sustain seabass farming year-round. The natural conditions in Chachoengsao Province, particularly the salinity of the Bang Pakong River, allow for seabass farming throughout the year. The farming process for giant seabass takes approximately 18-20 months, with the fish reaching a weight of over 5 kilograms, whereas in other regions, seabass farming typically produces smaller fish (700-900 grams) within 4-5 months.

The Bang Kluai group manages the entire seabass production cycle, from breeding to rearing the fish to their full size. The primary seabass breeding centers are located in Song Khlong Subdistrict and Tha Sa-an Subdistrict, both in Bang Pakong District, which are the largest seabass breeding areas in the region. The group includes breeders, hatchery operators, and those responsible for nurturing seabass from fry to full-sized fish. The resulting giant seabass exhibit several distinctive characteristics: 1) The fish are large, robust, with firm, white, and beautiful flesh that does not disintegrate during cooking and lacks a fishy odor. 2) They are highly nutritious, rich in Omega 3 and Omega 6, and contain brain-healthy nutrients directly sourced from natural water. 3) The fish have a delicious flavor, are fresh, clean, and free from chemicals, as no chemicals or antibiotics are used in the farming process. 4) The fish lack the typical fishy smell due to a blood-draining process, preserving the flesh's freshness and quality. 5) The filleted fish display a rainbow sheen (achieved by fat integration into the flesh, known as "aging"), resulting in tender, translucent flesh. 6) The scales are shiny and glossy, with the skin remaining white after descaling. The fish are long, with thick backs and firm flesh. The group has adopted the Japanese technique of “Ikejime” for bleeding the fish, which eliminates the fishy odor, preserves the flesh, and maintains a good texture. Additionally, the group employs unique processing techniques that enhance the flavor, sweetness, and texture of the flesh, resulting in a product with a distinctive rainbow sheen.

The production process of giant seabass in this region is divided into three main stages: 1) seabass fry production, 2) rearing the fish to a marketable size, and 3) raising the fish to their full giant size. Each stage is critical, starting with the preparation of broodstock, hatching, and nurturing the fry to produce high-quality seabass with good growth rates, high survival rates, and the appropriate size for farmers' needs. The process involves careful pond preparation, fry release, feed management, water quality control, harvesting of market-sized fish, and raising the fish to their full size as giant seabass. This comprehensive production cycle, from breeding to processing, ensures that the seabass produced meets high-quality standards, adding value to the product and enhancing the farmers' competitiveness in the market.

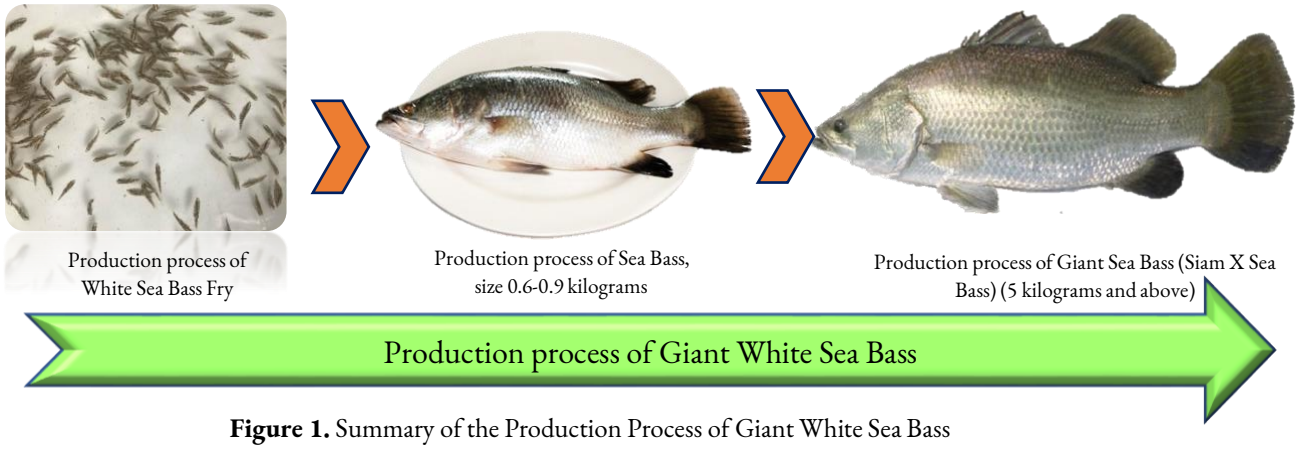

Figure 1. Summary of the Production Process of Giant White Sea Bass

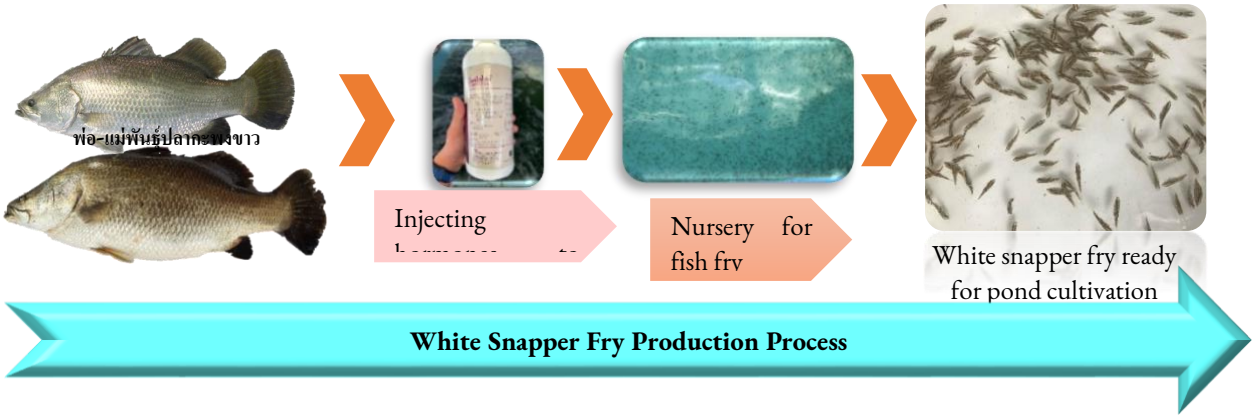

Figure 2. Summary of the White Snapper Fry Production Process

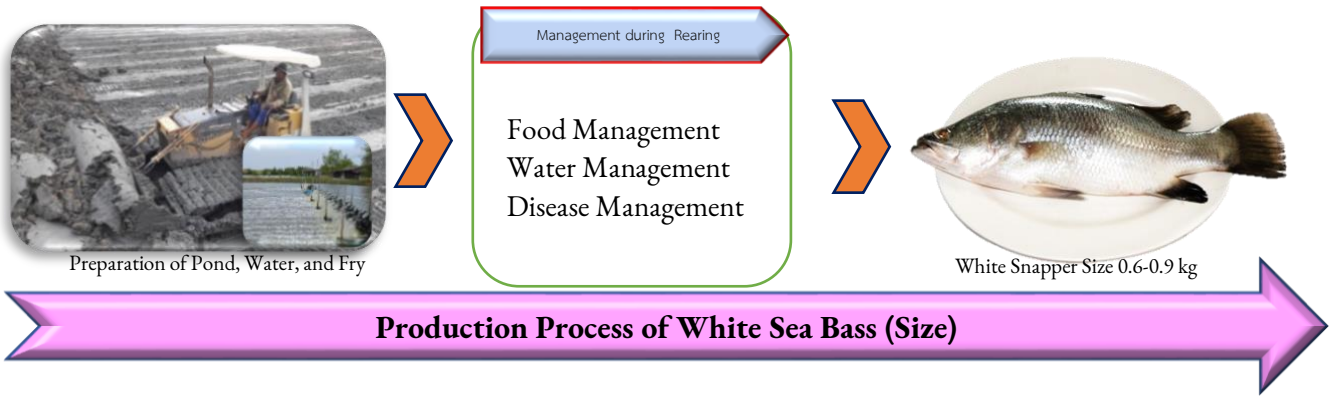

Figure 3. Summary of the White Sea Bass (Size) Production Process

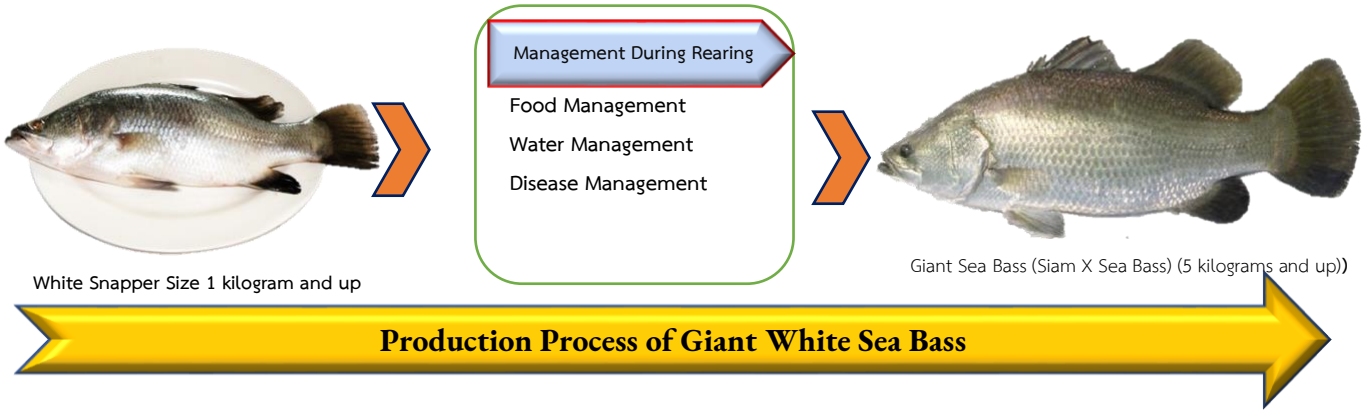

Figure 4. Summary of the Giant Sea Bass Production Process

Discussion and Conclusion

The key strengths of the giant seabass production by the producer group are rooted in the cultivation techniques. The seabass farmers possess significant expertise, effective farm management, superior fish breeds, excellent water quality, and substantial knowledge in rearing giant seabass. As a result, they produce giant seabass with the following characteristics: 1) The fish are large, robust, and strong with firm, white, beautiful flesh that does not disintegrate when cooked. The flesh is firm yet tender, does not break apart during cooking, and is free from any fishy odor. 2) The fish are highly nutritious, rich in Omega 3 and Omega 6, and contain nutrients beneficial for brain health due to the fish obtaining minerals directly from natural water sources. 3) The fish have a delicious flavor, are fresh, clean, and free from chemicals (as no chemicals or antibiotics are used in the farming process). 4) The flesh lacks any fishy smell due to the blood being effectively drained from the fish, maintaining freshness, white color, and preventing rapid deterioration. 5) The filleted fish exhibit a rainbow sheen (achieved by fat integration with the flesh, known as "aging"), resulting in tender, translucent flesh (weighing 1 kilogram). 6) The scales are shiny and glossy, with the skin remaining white after descaling (white and beautiful skin). The fish are long with thick backs (long and thick), and the flesh is firm (farmers believe this is due to the soil properties in the seabass farming area). Additionally, the producer group has adopted the technique of bleeding the fish, which effectively removes any fishy odor. The process, performed by skilled personnel using a method called “Ikejime” derived from Japanese practices for preparing fish for raw consumption, involves bleeding the fish through the gills. This technique eliminates any fishy odor, preserves the flesh for an extended period, and ensures the flesh remains white and translucent with a good texture. The processing of the fish is carried out using unique techniques by the farmers, resulting in flesh that is flavorful, sweeter, firmer, and exhibits a rainbow sheen (due to fat integration with the flesh, known as "aging"), a distinct characteristic of the giant seabass produced by the group.

The production process of giant seabass has been divided by the researcher into three stages: 1) the production process of seabass fry, 2) the process of growing seabass to a marketable size, and 3) the process of rearing seabass to become giant seabass. Each stage of the production process is critical, starting from the crucial steps of breeding and nurturing seabass fry, including the preparation of broodstock and the provision of food during the first 2-3 weeks after hatching. The majority of seabass used for breeding come from sources maintained by the Department of Fisheries. According to the Department of Fisheries (2001), the nurturing of seabass fry is divided into two phases: the first phase involves raising the fry from hatching until they are one month old, which is crucial for their survival. Subsequently, the fry are raised in cages until they reach a size of 3 centimeters or more, at which point they are ready for sale. The seabass fry farms are considered the primary production system in the large-scale giant seabass production chain, providing fry to members who require them, enhancing the efficiency of seabass farming. This system begins with nurturing the fry until they are ready for further farming, allowing members to farm throughout the year, reducing the time and improving the quality of farming for seabass farmers. The fish grow faster and are ready for sale more quickly. Since seabass have different eating behaviors from other fish, preferring fresh food and not eating leftovers, producers must understand this behavior to feed seabass correctly, which directly affects their growth. The Sriracha Fisheries Research Station (2003) noted that while feeding fish might seem simple, it actually requires an understanding of the principles, methods, and environmental changes in which the fish live, as well as the fish's habits and behaviors. These factors must be integrated to ensure that the fish eat well, make the most of their food, and minimize waste, as aquatic feed is a crucial factor in aquaculture production. The distinctive characteristics of giant seabass farming, unique to the area and reflective of the local identity, have led to a demand among farmers to elevate giant seabass as a provincial specialty product by registering it as a Geographical Indication (GI) product. This would establish it as a standard product, build consumer confidence in a product with unique characteristics, and increase farmers' income through the sale of standardized, unique products in global markets. The GI registration process requires thorough preparation of geographic and biological information, production factors, and management processes, with active participation from farmers and producers in every step to highlight the importance of collective involvement in the GI registration process.

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Research Article

Comparison of the growth of Spirulina sp. in feed formulas containing rice straw fermentation liquid as an ingredient at different levels

Arom Tiaosakul[1]*, Sayam Raso[2], and Sukjai Rattanayurakorn[3]

Fisheries Department, Faculty of Agriculture and Technology, Surin, Thailand

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Abstract

The objective of this research was to study the effects of different feed formulas on the growth of Spirulina and water quality in terms of temperature, pH and light intensity. The experiment was designed with completely randomized design (CRD), divided into 3 sets of treatments with different feed formulas with 3 replicates, using formula 1(Zarrouk's medium as control formula); formula 2(Zarrouk's medium mixed with 100 ml/1,000 ml of rice straw ferment liquid), and formula 3(Zarrouk's medium mixed with 200 ml/1,000 ml of rice straw ferment liquid) with 10 days cultured period provided with 24-hour light cycle. The growth efficiency was measured by measuring the average of Optical Density (OD), using a spectrophotometer at the wavelength of 560 nm. The results showed that the growth rate of Spirulina cultured with formula 1 was a statistically significantly different level of 95 % confidence ($p<0.05$) with, the growth rate of Spirulina cultured with formula 2 and 3. While the growth rate of Spirulina cultured with formulas 2 and 3 was not statistically different at a statistical significance level of 95 % confidence ($p>0.05$). In terms of water quality throughout the Spirulina culture, the temperature and pH values were in the range of 26.80±0.53 - 32.40±0.30 °C and 9.46±0.05 - 10.12±0.09, while the light intensity values were in the range of 1,125 - 1,950 lux, respectively

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Introduction

Rice straw is the part of the rice plant that remains after harvesting and removing the seeds. Rice straw contains the following primary and secondary nutrients: nitrogen (N) = 0.59 %, phosphorus (P) = 0.08 %, potassium (K) 1.56 %, calcium (Ca) 0.38 %, magnesium (Mg) 0.23 % and sulfur (S) 0.08 % (Terra-Gro Fertilizer Co., Ltd., 2023) It contains protein, dietary fiber and phosphorus (Spring Green Evolution Co., Ltd., 2023) total carbon content of 39.2 %, calcium 262 mg kg^{-1} and sodium 366 mg kg^{-1} (Abdelhamid, 2004). These elements are important for plant growth. Farmers generally use rice straw for agricultural purposes, such as as mulch to retain moisture, for animal feed, for compost or for plowing to increase organic matter which is a source of food and energy for beneficial microorganisms in the soil. In the fisheries sector, farmers use it as a food source for fish or as a fish food sandwich. By using rice straw as the main base

1 Corresponding Author: Fisheries Department, Faculty of Agriculture and Technology, 145 Moo 15, Surin-Prasart Road, Nokmuang , Surin, Thailand. E-mail: apermngam@gmail.com
2 Science in Creative biology, Faculty of Science and Technology, Surindra Rajabhat University, 186 Moo 1, Surin-Prasart Road, Nokmuang, Surin, 32000 , Thailand.
3Science and Mathematics Department, Faculty of Agriculture and Technology, 145 Moo 15, Surin-Prasart Road, Nokmuang, Surin, 32000, Thailand.

and placing it alternately with animal feces or other manures at the corners of the pond to provide a food source for the fish in the pond. Some farmers throw rice straw into the fish pond for about 3-5 days. The straw soaked in water will gradually decompose. Nile tilapia and other herbivorous fish such as carp, gourami, and striped catfish, etc., will eat the decompose rice straw until it is all gone. In addition to being food for herbivorous fish, rice straw soaked in water is also a hiding place and helps create algae and plankton, which are another type of fish food. In addition to helping solve the problem of smog caused by burning rice straw, it can also help save on fish farming costs and make fish meat firm and taste sweet like fish in natural water sources (Sridarat, 2012).

Spirulina is a blue-green algae, It is a single-celled algae that has a spiral shape. It is found in freshwater natural water sources, from cultured areas, and grows well in both clean and wastewater (Wongrat, 1996). This type of algae has a very high protein level, so it has received attention and is used as a supplement for health-conscious people and mixed in aquaculture formula feed (Bhattacharya and Shivaprakash, 2005) or used to replace protein sources from other sources (Boonsom, 1992). Spirulina contains a high protein content of 50-70 percent of dry cell weight and contains other important substances such as Phycocyanin, Allophycocyanin, Beta-carotene, Chlorophyll-a and unsaturated essential fatty acids (Vekataraman, 1983).

Most spirulina farming uses chemical fertilizers as a food source, which has high production costs, as an alternative to use as a supplement in ready-made aquatic animal feed with high production capacity, and to reduce the cost of cultivation, by finding natural food sources such as fermented animal waste or fermented plants (Chanchay and Chudarat, 2018).

Therefore, from the reasons mentioned above, to be a guideline to reduce the cost of spirulina cultivation with fermented water from rice straw, which is a plant residue left over from agriculture after harvesting, the data obtained from the study will be basic information to be applied to reduce the production cost of spirulina and can apply it to create benefits in the future.

Aim of the Study

This study aims to determine the effects of different feed formulas of the growth of *Spirulina* sp. in feed formulas containing rice straw fermentation liquid and water quality in terms of temperature, pH and light intensity.

Method

Preparation of water for culturing Spirulina algae Use tap water that has been left in a 50-liter plastic tank for 5 days and filter it with a thin cloth before using it in the experiment.

Preparation of Spirulina algae Spirulina algae, which was kindly provided by the Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai Province, was cultivated in the laboratory using Zarrouk's medium to prepare for use in the experiment. Before using it, the algae were measured for cell density (Optical Density; OD) using a Spectrophotometer at a wavelength of 560 nanometers, to obtain an Optical Density (OD) value in the range of 0.80-0.90 for use in the study (adapted from Chuchet et al., 2010).

Preparation of rice straw fermentation solution Cut rice straw into small pieces and ferment with prepared tap water in a ratio of rice straw: water with ratio 1:10. Leave to ferment for 4 weeks.

Preparation of the control formula (Zarrouk's medium) adapted from Meng-Amphan and Nokham (2007) the solution consists of the following Sodium bicarbonate ($NaHCO_3$) 4.80 grams, Sodium hydroxide (NaOH) 1.12 grams, N-P-K fertilizer (16-16-16 formula), and Magnesium sulphate ($MgSO_4$) 1.62 grams respectively. These chemicals were dissolved in 600 milliliters of tap water. This process was repeated in 9 glass jars, each with a capacity of 3,000 milliliters, to prepare for the experiment.

The Completely Randomized Design (CRD) trial was planned, divided into 3 sets of 3 replicates each, as follows.

- Treatment 1 (T1) Feed formula 1 used Zarrouk's medium (control formula)
- Treatment 2 (T2) Feed formula 2 used Zarrouk's medium formula and 100 ml of rice straw fermentation liquid

- Treatment 3 (T3) Feed formula 3 used Zarrouk's medium formula and 200 ml of rice straw fermentation liquid

Add 200 ml of spirulina to every glass jar prepared above and adjust the water level in each glass jar to 1,000 ml with the prepared tap water. Place the glass jars in random positions, install an aeration set, and provide lighting with 120 cm long LED bulbs in every row of the glass jars throughout the 10 days.

Data collection and analysis

Water quality analysis analysed before the experiment and every day of algae cultivation, including pH and temperature (**°C**) using a multifunctional water analyzer model 9909 SP.

Light intensity by randomly measuring the light intensity at 3 points in the experimental area that received light from electric bulbs while growing spirulina algae by using the TECPEL 530 light intensity meter.

Optical Density (OD) measurement was modified from Promya and Saetan (2005) by randomly collecting algae samples from each experimental set using a Spectrophotometer (Jasco Model V-530, Japan) at a wavelength of 560 nm every day throughout the 10-day cultivation period to compare the algae growth rate (Growth rate, percentage; %) at a wavelength of 560 nanometers every day throughout the 10-day cultivation period to compare the algae growth rate (Growth rate, percentage; %) which is calculated by the following equation:

$$\text{Growth rate (\%)} = \frac{(OD_i - OD_o) \times 100}{OD_o}$$

When

OD_o = OD value of algae on day 0

OD_i = OD value of algae harvested on day i (i = 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10)

Data analysis of the growth rate and water quality data was analysed using the One-way analysis of variance (ANOVA) method according to the randomized experimental plan and the differences in the mean values were compared using the Honestly Significant Differences (Tukey's Test HSD) method at a 95 percent confidence level using the IBM SPSS Statistics V.26 ready-made program. The light intensity values will be reported as the mean values.

Results

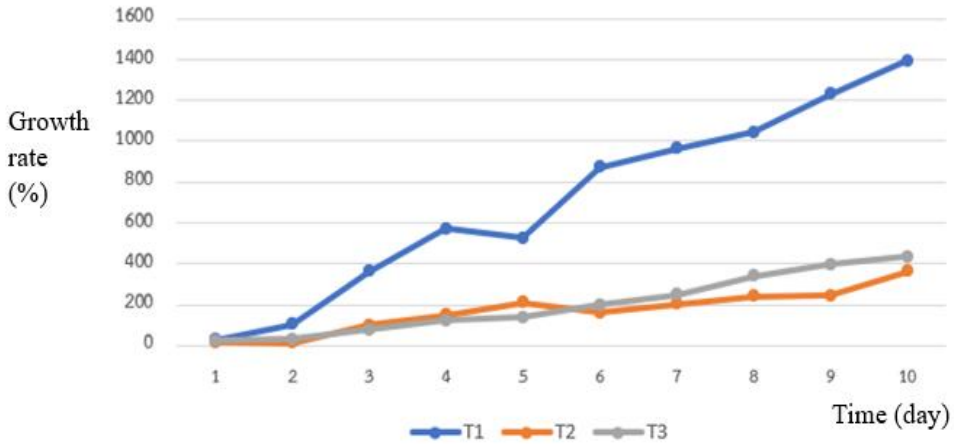
The results of the study comparison of *Spirulina* sp. cultivation yields in feed formulas containing rice straw fermentation as an ingredient at different levels showed that the growth rate of Spirulina algae with Feed formula 1 (T1) had an average growth rate of 9.46±0.05, 101.21±137.89, 363.53±125.26, and 573.05±198.01, 525.26±198.01, 871.27±176.37, 963.67±173.52, 1,044.51± 321.19, 1,228.52± 463.03 1,394.58±558.91 % on day 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, Feed formula 2 (T2) had an average growth rate of 15.04±28.66, 12.54±81.42, 100.07±68.76, 148.63±95.30,208.77± 137.50, 159.44±116.32, 201.19± 130.79, 240.64±193.23, 245.11± 135.44 and 361.42±201.54 % on day 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, Feed formula 3(T3) had average growth rates of 22.19± 33.41, 31.80±20.42, 77.94±89.52, 120.85± 107.84, 136.19±85.22, 197.60±124.39, 247.65±150.64, 338.00± 338.00±193.93, 395.53 ± 207.59 and 435.34± 238.54 % on day 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, respectively (Table 4.1 and Figure 4.1). The growth rate of Spirulina algae after 10 days, Spirulina, algae cultured with Feed formula 1(T1) tended to have the highest growth rate (1,394.58±558.91 %) followed by Spirulina algae cultured with Feed formula 3 (T3) (435.34± 238.54 %) and 2 (T2) (361.42± 201.54 %), respectively.

When the growth rate data were analysed statistically, it was found that the growth rate of Spirulina cultured with Feed formula 1 was significantly different from the growth rate of Spirulina cultured with Feed formula 2 and 3 at a statistical significance of 95 % confidence level ($p < 0.05$). However, the growth rate of Spirulina cultured with Feed formula 2 and 3 was not statistically different at a statistical significance of 95 % confidence level ($p > 0.05$). (Table 4.1).

Table 1. Shows the average growth rate (%) of Spirulina algae over a period of 10 days

Days	Feed formula		
	1 (T1)	2 (T2)	3 (T3)
1	9.46±0.05[a]	15.04±28.66[b]	22.19±33.41[b]
2	101.21±137.89[a]	12.54±81.42[b]	31.80±20.42[b]
3	363.53±125.26[a]	100.07±68.76[b]	77.94±89.52[b]
4	573.05±198.01[a]	148.63±95.30[b]	120.85±107.84[b]
5	525.26±198.01[a]	208.77±137.50[b]	136.19±85.22[b]
6	871.27±176.37[a]	159.44±116.32[b]	197.60±124.39[b]
7	963.67±173.52[a]	201.19±130.79[b]	247.65±150.64[b]
8	1044.51±321.19[a]	240.64±193.23[b]	338.00±193.93[b]
9	1228.52±463.03[a]	245.11±135.44[b]	395.53±207.59[b]
10	1394.58±558.91[a]	361.42±201.54[b]	435.34±238.54[b]

Note: Identical letters on the same line are not statistically different at 95 % confidence.



Picture 1. The average growth rate (%) of Spirulina algae over a period of 10 days

Water quality in terms of acidity-alkalinity (pH) during from the beginning to the end of the experiment 10 days in the glass jars of all treatments, the results showed that the average pH values in Feed formula 1(T1) were 9.46±0.05, 9.67±0.03, 9.96±0.06, 9.70±0.02, 9.90±0.0, 9.79±0.03, 9.88±0.12 10.00±0.14, 10.01±0.23, 10.07±0.20 and 9.91±0.22; 9.51±0.05, 9.70±0.07, 9.65±0.06, 9.68±0.02, 9.81±0.05, 9.78±0.05, 9.91±0.05, 10.03±0.07, 10.00±0.15, 10.12±0.09 and 9.97±0.12 in Food formula 2(T2) and 9.58±0.01, 9.65±0.03, 9.70±0.02, 9.68±0.02, 9.81±0.02, 9.74±0.01, 9.86±0.04, 9.86±0.03, 9.81±0.08, 9.93±0.06 and 9.90±0.02 in Feed formula 3(T3), respectively (Table 2 and Figure 2 A).

The average pH value of all treatments was between 9.46±0.05 - 10.12±0.09, and when the data were analyzed statistically, it found that all treatments were not statistically different at 95 % confidence level ($p > 0.05$) (Table 2).

Table 2. Shows the average pH values during the cultivation of Sprulina algae with different formulas for 10 days

Days	Feed formula		
	1 (T1)	**2 (T2)**	**3 (T3)**
1	9.67±0.03[a]	9.70±0.07[a]	9.65±0.03[a]
2	9.96±0.06[a]	9.65±0.06[a]	9.70±0.02[a]
3	9.70±0.02[a]	9.68±0.02[a]	9.68±0.02[a]
4	9.90±0.06[a]	9.81±0.05[a]	9.81±0.02[a]
5	9.79±0.03[a]	9.78±0.05[a]	9.74±0.01[a]
6	9.88±0.12[a]	9.91±0.05[a]	9.86±0.04[a]
7	10.00±0.14[a]	10.03±0.07[a]	9.86±0.03[a]
8	10.01±0.23[a]	10.00±0.15[a]	9.81±0.08[a]
9	10.07±0.20[a]	10.12±0.09[a]	9.93±0.06[a]
10	9.91±0.22[a]	9.97±0.12[a]	9.90±0.02[a]

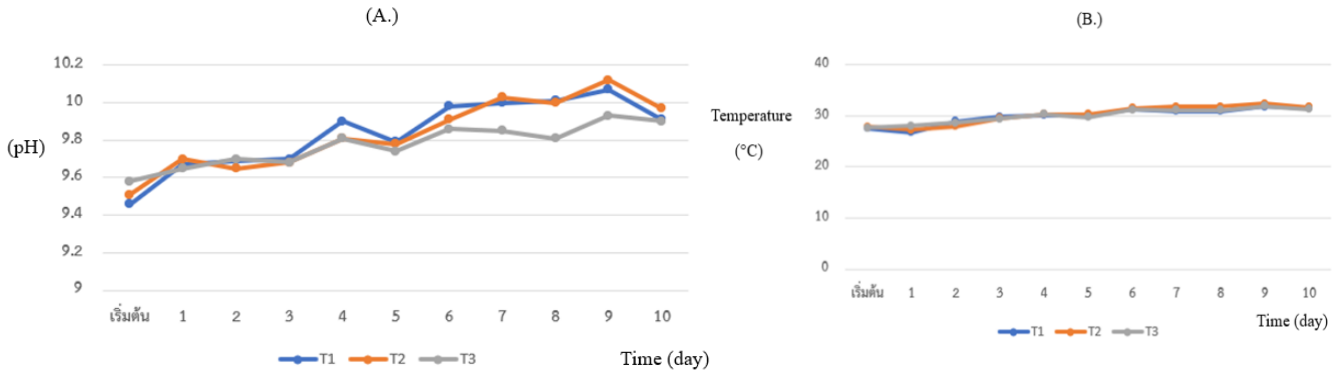
Note: Identical letters on the same line are not statistically different at 95 % confidence

For water quality in terms of temperature (°C) from the beginning to the end of the experiment 10 days in the glass jars of all treatments, the results showed that the average temperature values in Food formula 1(T1) were 27.57±0.47, 26.80±0.53, 28.93±0.31, 29.87±0.32, 30.10±0.17, 30.07±0.06, 31.30±0.56, 30.97±0.38, 30.97±0.38, 31.77±0.47 and 31.47±0.47 °C; 27.90±0.44, 27.27±0.31, 28.00±1.00, 39.57±0.21, 30.33±0.3,5 30.30±0.26, 31.50±0.87, 31.83±0.25, 31.83±0.25, 32.40±0.30 and 31.70±0.60 and 27.67±0.12, 28.03±0.38, 28.60±0.60, 29.43±0.65, 30.33±0.57, 29.77±0.75, 31.33±0.31, 31.10±0.53, 31.10±0.53, 31.90±0.72 and 31.30±0.92°C in Feed formula 3(T3), respectively (Table 3 and Figure 2 B). All treatments' average temperature (°C) values were between 26.80±0.53 and 32.40±0.30°C. When the data were analyzed statistically, it was found that all treatments were not statistically different at a 95 % confidence level ($p > 0.05$) (Table 3). While the average light intensity was in the range of 1,125 - 1,950 lux.

Table 3. Shows the average temperature (°C) values during the cultivation of Sprulina algae with different formulas for 10 days

Days	Feed formula		
	1 (T1)	**2 (T2)**	**3 (T3)**
1	26.80±0.53[a]	27.27±0.31[a]	28.03±0.38[a]
2	28.93±0.31[a]	28.00±1.00[a]	28.60±0.60[a]
3	29.87±0.32[a]	39.57±0.21[a]	29.43±0.65[a]
4	30.10±0.17[a]	30.33±0.35[a]	30.33±0.57[a]
5	30.07±0.06[a]	30.30±0.26[a]	29.77±0.75[a]
6	31.30±0.56[a]	31.50±0.87[a]	31.33±0.31[a]
7	30.97±0.38[a]	31.83±0.25[a]	31.10±0.53[a]
8	30.97±0.38[a]	31.83±0.25[a]	31.10±0.53[a]
9	31.77±0.47[a]	32.40±0.30[a]	31.90±0.72[a]
10	31.47±0.47[a]	31.70±0.60[a]	31.30±0.92`[a]

Note: Identical letters on the same line are not statistically different at 95 percent confidence



Picture 2. Water quality in terms the average of acidity-alkalinity (pH): (A.) and the average temperature (°C): (B.) during the cultivation of Sprulina algae with different formulas for 10 days

Conclusion and Discussion

From the study of Comparison of the Growth of Spirulina sp. in feed Formulas Containing Rice Straw Fermentation Liquid as an Ingredient at Different Levels for 10 days, the results showed that the growth rate of Spirulina sp. cultured by feed formula 1 using Zarrouk's medium (control formula) was statistically different at 95 % confidence ($p<0.05$) with, Spirulina cultured by feed formula 2 (using Zarrouk's medium and 100 ml of rice straw fermentation liquid) and feed formula 3 (using Zarrouk's medium and 200 ml of rice straw fermentation liquid). While the growth rate of Spirulina cultured by feed formulas 2 and 3 was not statistically different at a statistical significance level of 95 % confidence ($p>0.05$).

A study of Tansakun and Chanthasin (1988) investigating the supplementation of certain primary nutrients, specifically $NaNO_3$ at various concentration levels, in wastewater from rubber factories yielded notable results. The growth rate of Spirulina algae reached its peak when $NaNO_3$ was added at a concentration of 2.5 grams per liter, surpassing the growth rate observed with standard nutrient media. Conversely, the addition of K_2HPO_4 and K_2SO_4 did not significantly impact the growth rate of Spirulina algae. In fact, increasing the concentration of these compounds to high levels resulted in a decrease in growth rate.

Furthermore, a comparative analysis was conducted between two nutrient combinations: $NaNO_3$ at 2.5 grams per liter combined with K_2HPO_4 at 0.1 grams per liter, and $NaNO_3$ at 2.5 grams per liter combined with K_2SO_4 at 0.1 grams per liter. The results indicated that both combinations yielded lower Spirulina growth rates compared to the standard Zarrouk's medium. A notable observation from this study was the correlation between nutrient solution pH and Spirulina growth rates. Nutrient formulations that promoted high growth rates in Spirulina were characterized by high pH values. Conversely, nutrient formulations associated with decreased Spirulina growth rates exhibited low pH values.

Laophongphich (1989) experimented with the cultivation of Spirulina (*S. platensis*) in distillery wastewater at concentrations of 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 percent. The medium was supplemented with chemical additives: 8.5 g/L $NaHCO_3$, 1.5 g/L NaNO3, 0.5 g/L K2HPO4, and 0.6 g/L N-P-K fertilizer (16-16-16 formula). The pH was adjusted to 10 ± 0.1, and the cultivation period lasted 24 days under both laboratory and outdoor conditions. The results indicated that in both environments, the algae exhibited optimal growth at a distillery wastewater concentration of 0.5 %.

Ciferri (1983); Venkataraman (1983) and Nakamura (1982) reported the pH levels observed during Spirulina cultivation of all nutrient formulations averaged between 9.46±0.05 and 10.12±0.09, which falls within the optimal range for Spirulina growth. As with the experiment of Chuchet et al. (2010) indicates that the ideal pH range for Spirulina growth is between 9.5 and 10.5, and Vincent and Silvester (1979) reported that Spirulina thrives in pH conditions ranging from 9 to 11, as this range facilitates optimum nutrient availability for the cyanobacterium growth and pH levels are closely related to the dissolution of carbon dioxide (Venkataraman, 1983) or the concentration of bicarbonate ions (Bunnak, 1986). These factors directly and indirectly influence the metabolic processes of algal cells

(Becker and Venkataraman, 1982). The pH levels affect photosynthetic processes and the activity of various enzymes involved in algal photosynthesis. But, in the present study, the pH levels ranged from 7.30 to 8.30, which is below the optimal range for Spirulina growth. While the temperature ranged from 26.80±0.53 to 32.40±0.30 °C. This temperature range is conducive to optimal Spirulina growth (Venkataraman, 1983).

The wavelengths that Spirulina can utilize photosynthesis are identical to those used by typical plants (Trissl, 1993). Increased light intensity enhances photosynthesis and accelerates cellular activity (Niangoran et al., 1974). When the light intensity is below 2000 lux, algae, and higher plants can only utilize approximately 20 % of the incident light energy (Bunnak, 1986). The algae's energy utilization efficiency decreases at light intensities exceeding 8000 lux. And, Nakamura (1982) suggests that the optimal light intensity for Spirulina growth is between 4,000-5,000 lux, while Tansakun and Chanthasin (1988) report a requirement of 3,500 lux for 16 hours per day. But, in the present study, throughout the experiment, the light intensity ranged from 1,125 lux to 1,950 lux., which is below the range for Spirulina growth as mentioned above.

Recommendations

Based on the above findings and conclusion, the following are the study's recommendations:

- In the cultivation of Spirulina using rice straw fermentation extract, it is suggested to supplement the culture medium with certain essential macronutrients, such as $NaNO_3$ and pH optimal range. This compound not only serves as a nutrient source for Spirulina, but also aids in elevating the pH level to a range between 9 and 11. This pH range is optimal for nutrient utilization, significantly impacting photosynthetic processes and the activity of various enzymes involved in algal photosynthesis.
- For optimal Spirulina cultivation, it is recommended to utilize areas with high light intensity or outdoor locations where illuminance exceeds 1,950 lux. The ideal light intensity range for cultivation is between 4,000 and 5,000 lux. To exceed this range can occur the photoinhibition.
- Rice straw, an agricultural by-product remaining after harvest, can be utilized in small quantities to produce fermentation extract. This application presents a potential avenue for reducing production costs in Spirulina cultivation in the future, offering an innovative approach to sustainable algal biotechnology in rural area application.

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Research Article

Wheat leaf rust disease caused by Puccinia triticina Eriks

Şerife Nergis Bayar[1]

Plant Protection Department, Aegean Agricultural Research Institute, Menemen, Türkiye

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Abstract

One of the biggest obstacles to increasing wheat production and yield is diseases. Diseases not only reduce the amount of product but also negatively affect product quality. Rust diseases in wheat are among the most important biotic stress factors limiting wheat production in our country. In epidemic years, early infections on cereals susceptible varieties can cause yield losses of up to 80-90% and can cause varieties to be completely removed from production. In our country, yield losses caused by different types of rust on wheat plants have been recorded between 12-80%. Product losses vary depending on the sensitivity of varieties, environmental conditions, and the races of the factors, as well as from year to year and from region to region. Leaf rust (Lr) (Puccinia triticina Eriks.) is seen almost everywhere wheat is grown, but the damage it causes is not as noticeable as the damage caused by black and yellow rust. However, these two rust agents are not effective every year and cause serious epidemics every 7-8 years. Leaf rust, on the other hand, occurs almost every year and causes a certain amount of yield loss. The most important method for creating broad-spectrum disease resistance in cereals is the combined use of biotechnology and traditional breeding methods.

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Introduction

The grain group is very important in terms of agricultural production as well as agriculture and food industry. When industry is involved, it is important in both of labor force, value-added product, economy and environmental safety. Both in other countries in the world and in Türkiye wheat is more important than other agricultural products as it is the raw material of the most basic nutrients in human nutrition. As it is known that, wheat has strategic importance as a basic food source in the world. It is especially the most suitable and cheapest grain for bread making. In addition flour, which has more economic value, is obtained from wheat and the remaining part is used as animal nutrition (Anonymous a., 2024). When we look at wheat cultivation areas around the world the top 5 countries are India, Russia, China, EU and USA. Cereals have the largest cultivation area and production among the cultivated plants in the world. Türkiye's wheat cultivation area constitutes 3.5% of the world's wheat cultivation area, and this area also corresponds to 33% of the total cultivated agricultural area in Türkiye (Anonymous b., 2024) (Table 1).

1 PhD., Plant Protection Department, Aegean Agricultural Research Institute, Menemen, Türkiye. E-mail: serifenergis.celik@tarimorman.gov.tr

Table 1. Türkiye Wheat Production by Regions in 2022.

Region Names	Cultuvated area (hectar)	Production	Yield
Marmara Region	850.000	3.800.000	4.47
Aegean Region	650.000	1.650.000	2.54
Central Anatolia Region	2.200.000	5.250.000	2.39
Mediterranean region	600.000	1.600.000	2.67
Eastern Anatolia Region	650.000	1.500.000	2.31
Southeastern Anatolia Region	1.100.000	2.200.000	2.00
Black Sea region	610.000	1.500.000	2.46
Total	6.660.000	17.500.000	2.63

Among the organisms that cause diseases in cereals, pathogenic fungi are one of the most important diseases that can be found in almost every region in our country. Among these, rust diseases stand out with their serious yield losses. Since rust become the most widespread and important diseases of wheat, they started to be studied more. Wheat rust resistance gene resources have been used by breeders for many years and therefore new breeds that can break the effects of the resistance genes of host plants can emerge. Genetic resistance is the cheapest and most effective method for controlling rust diseases although protective fungicides are recommended against this diseases, it is not economical in practice. Therefore, in addition to cultural measures, using resistant varieties or lines is the most effective control method (Anonymous c., 2024).

Resistance to pathogens in host plants;

Race-specific (vertical) resistance: A host plant while it is resistant to some physiological races of the pathogen, it is sensitive to other races of the pathogen. Race-specific resistance breaks down quickly. However, this type of resistance provided by a few large genes which is not a very desired type of resistance, as it is easily broken by high pathogen populations in environment and the emergence of new races. Therefore, in order to control newly developing virulent pathogen races, breeders are constantly creating new varieties with new resistance genes or gene combinations must be developed.

Non-race specific (horizontal) resistance: It shows resistance against all races of the host pathogen. Many moderately effective genes or their interactions play a role in this type of resistance.

In wheat production areas, rust (Puccinia spp.) diseases are very important and can cause big yield and quality losses at fields. The climate factor is also critical for rust diseases. It is known that the occurrence of this diseases and the magnitude of the damage also depend on the virulence pattern of the disease population and the host genotype (Singh et al., 2004a).

Taxonomy of Puccinia spp. is; Kingdom: Fungi, Phylum: Basidiomycota, Class: Urediniomycetes, Team: Uredinales, Family: Pucciniaceae, Genus: Puccinia

Puccinia spp. urediospores, which play an important role in spread of rust diseases, are mostly formed in spring and summer. They carried out by wind and cause new infections in other plants. In temperate zones, the rust spores spend winter as urediospores in autumn on crops and wild grasses. Summer spores, which are formed in spring and spread around by wind and cause new infections in optimum humidity and temperature (10-18°C) conditions (Lipps, 2006). Urediospores are single-celled. The fungus enters the host plant through stomata by being transmitted to the host plant by external factors such as wind. Urediospores which are on the leaf surface absorb water and swell when they come into contact with rain or dew, developing germination tubes. This germination occurs 4-8 hours later at 20 °C under 100% humidity, and the spores can maintain their viability for 1-3 days without germination (Bolton et al., 2008).

There are 3 rust diseases on wheat. Yellow (stripe) rust (*Puccinia striiformis* f.sp. *tritici*), stem rust (*Puccinia graminis* f. sp. *tritici)* and leaf rust (*Puccinia triticina* Eriks.). Since pesticide treatment is often economical in the fight against this diseases, the use of resistant varieties are important when climate conditions are optimum for disease development, rainfall and temperature is effective (Bayram et al., 2007). Yellow rust is an epidemic disease and causes more crop losses

in coastal areas and mid-elevation mountainous regions with cool and rainy climates. The disease, which spreads unnoticed for a few years, can suddenly turn into an epidemic causing significant losses.

Stem rust is seen towards the end of the season and causes significant crop losses in humid conditions and high temperatures. It is known that it causes 50-80% yield losses in many countries (Singh et al., 2004b). Leaf rust disease occurs more regularly each year compared to the other two rust diseases seen in wheat (Chester, 1946; Samborski, 1985; Aykut Tonk and Yüce, 2007). The damage caused by leaf rust on wheat depends on the wheat's growth period at the time of disease infection in relation to the development phase of the rust. Serious losses occur before or during flowering and especially when the flag leaf is infected. Especially in last 30 years, the widespread use of rust-resistant winter wheat varieties have been effective in reducing losses caused by leaf rust. There are many races of leaf rust fungus and varieties are not resistant to all races. New races occur every few years and previously resistant varieties become susceptible. The resistance period of varieties resistant to leaf rust generally remain between 2-4 years (Lipps, 2006). Leaf rust causes approximately 50% yield losses in wheat worldwide. Although it does not cause large and sudden product losses. Yellow and black rust can cause more damage and yield losses. Because the disease is seen more or less every year and can cause significant crop losses over many years. In our country, leaf rust disease is mostly seen in the Aegean and Mediterranean coastal areas, and it also occurs in inland regions as the weather gets warmer. The countries and regions where leaf rust is most common are the USA, Canada, Western Europe, Eastern Russia, Siberia, China, South America, North Africa, India, Japan, Australia and Scandinavia (Altay, 1978; Liu and Kolmer, 1997).

Leaf rust (*Puccinia triticina* Eriks.) Disease Symptoms

Usually on leaf; under optimum conditions, infection can also occur in glumes and awns. Symptoms can also be seen on the leaf sheath and stem. Yield loss occurs in decrease in the number of grains per ear, a decrease in grain size, a decrease in 1000 grain and hectoliter weight and a decrease in protein content (Aktaş, 2001). This disease generally limits the photosynthesis area with the pustules that it forms on the leaves (Khan et al., 1997). On the upper surface of the leaf, initially small, round to oval yellow spots appear on infected tissues. In late stages of the disease, these spots turn into orange coloured pustules and these pustules are surrounded by a yellow halo. These pustules form many spores that can be easily spread around. At late stages of the disease black spores can also form, also orange and black spores may appear together on the same leaf. Sometimes uredospore beds may occur as large beds in the middle and small beds at the edges. This look makes leaf rust identification easier (Singh et al., 2004a).

Main hosts of *Puccinia* spp.:
Wheat species: *Triticum aestivum* L., *T. tingidum, T. dicoccon*, T. *dicoccoides, Aegilops speltoides* and triticale.
Intermediate hosts: *Thalictrum flavum glaucum, Isopyrum fumaroides*.

Method of Determination of Wheat Leaf Rust Breeds (classical racial discrimination)

The wheat varieties which are available today are not mostly resistant to leaf rust. New races appear every few years and by the time resistant varieties become susceptible. Nowadays number of resistance genes are increased significantly. Leaf rust detected and mapped on wheat more than ever during the last 10 years (Bolat et al., 1999). The durability period of leaf rust resistant variety generally varies between 2-4 years. There are many races of leaf rust and the available varieties are not resistant to all races. By reforming of new races, winter spores (teliospores), which are necessary for the fungus to survive in unsuitable conditions, initiate the sexual period on intermediate hosts and enable the formation of new physiological races (Khan et al., 1997). The reasons for using different sets and genotypes in determining physiological races are explained by McIntosh et al. (1995) explained as follows;

- Although the virulence of the rust factor depends on a number of conditions, great changes can be seen depending on the different areas and years in which wheat is grown.
- Rusts can spread over long distances with the wind and getting effective in different areas. This situation increases race diversity in areas where the rust factor may be effective, and in order to define this diversity, the number of genotypes in the differential sets must be increased or expanded by adding new genotypes to these sets over time.

Although it seems possible to work with all defined genotypes although each of which has separate resistance genes, the need for a climate chamber/greenhouse, labor and other costs increases for race identification studies to be carried out with a large number of isolates. Identification of leaf rust races is carried out by seedling tests. Leaf rust 'Thatcher Monogenic Lines' consisting of 20 different genotypes are used as a breed differential set. Each of these lines carries different leaf rust resistance genes. In racial analysis, it is necessary to ensure that the spores are pure. To obtain pure spores, it is very important to start working with single pustule isolation. A differential set is used for each isolate that is sure to be pure. Thatcher Monogenic Lines are in groups of 4 and pure spore suspensions are inoculated to the leaves of 20 different Lr lines. The genotypes in the race-differentiating set are grown in pots and when the second leaf begins to appear, the isolates are The inoculation process will be carried out. Following inoculation the plants are kept in the dark at 18°C and 95% relative humidity for 24 hours and then transferred to climate chamber conditions at 15-20°C. Approximately 14 days after inoculation, scorings can ben done. The scale determines resistant and susceptible genotypes by using 0-4 scale (Stakman et al., 1962) based on infection types. According to this scale, 0, ;, 1, 2 and their combinations are resistant (R), 3-4 and their combinations are considered susceptible (S) genotypes (Table 2).

Table 2. Seedling test scale to determine wheat leaf rust races (Stakman et al., 1962).

Scale		Infection type
0	**Resistant**	There are no macroscopic signs of uredia or infection.
;	**Resistant**	There are no uredia, but necrotic chlorotic spots of different sizes belonging to hypersensitive reaction are seen.
1	**Resistant**	There are small uredia surrounded by necrotic areas.
2	**Resistant**	Small uredia surrounded by necrotic and chlorotic areas are mostly seen.
3	**Susceptible**	Medium-sized uredia with chlorosis, rarely necrosis are seen.
4	**Susceptible**	Large uredia without chlorosis or necrosis are seen.

Breed naming is done according to the North American standard breed naming system using 'Thatcher Isogenic Lines' (Long and Kolmer, 1989), (Table 3). In race diagnoses, each code is determined according to the infection types given by the 20 Lr line, and these codes are brought together to create codes and breeds are determined.

Table 3. *Puccinia* classification of different North American hosts for P. triticina spp. listed in groups of 4 (Long and Kolmer, 1989).

Thatcher Isogenic Lr Genes That Give Infection Types				
Wheat set 1	1	2a	2c	3a
Wheat set 2	9	16	24	26
Wheat set 3	3ka	11	17	30
Wheat set 4	b	10	14a	18
Wheat set 5	3bg	14b	20	28
***Pt* code**				
B	R	R	R	R
C	R	R	R	S
D	R	R	S	R
F	R	R	S	S
G	R	S	R	R
H	R	S	R	S
J	R	S	S	R
K	R	S	S	S
L	S	R	R	R
M	S	R	R	S
N	S	R	S	R
P	S	R	S	S
Q	S	S	R	R
R	S	S	R	S
S	S	S	S	R
T	S	S	S	S

Scoring of adult plant disease observations under field conditions, Modified It is made according to the infection severity range (0-100%) according to the Cobb Scale (Roelfs et al., 1992).

Table 4. Wheat rust disease field adult plant scale (Roelfs et al., 1992).

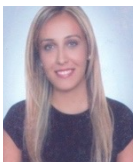
Reaction	Explanation
R	Resistant; necrotic spots with hollow or small pustules.
MR	Intermediate resistant; small pustules surrounded by necrotic areas.
MS	Intermediate sensitive; medium-sized pustules, no necrotic areas.
S	Sensitive; large pustules, necrotic spots, no chlorosis.

R: Resistant; MR: Moderately resistant; MS: Moderately susceptible; S: Susceptible

Conclusion

Wheat rust disease spread to all regions of our country where wheat is produced.The damage caused by rust disease in wheat varies according to climate changes, can sometimes cause epidemics and cause big damage. Yield loss according to susceptibility of varieties, environmental conditions and the races of the pathogens, as well as from year to year from region to region. The disease can spread rapidly over long distances with wind and human factors and the capacity to create new races races in the biological process of the disease multiplies the potential threat to wheat production on a global level. Factors such as the host's reaction to the disease and the phenological stage of the plant when the disease is first seen can affect the possible yield losses to varying degrees. In wheat, leaf rust disease caused by *P. triticina* is commonly seen in the coastal areas of Turkey. In epidemic years, yield losses caused by the disease are considerable. The most economical and environmentally friendly method to minimize yield losses caused by leaf rust disease is to use disease-resistant varieties. Leaf rust significantly affects grain quality and as well as yield. It reduces photosynthesis and increases respiration and evaporation. Thus, it significantly affects the development of the plant. In breeding studies to develop varieties resistant to leaf rust disease in wheat, classical breeding methods were used to crossbreed varieties with different resistance genes and to combine the resistance genes in a single variety. In recent years, DNA studies have become more important. With the development of molecular markers, marker-supported selection studies have accelerated and thus, studies on yield, quality, and resistance to diseases and pests in cereals have increased and successful results have been obtained. Using molecular biotechnology methods in combination with classical breeding methods in the development of varieties resistant to leaf rust disease saves time, labor and cost.

Biodata of Author



PhD. **Şerife Nergis Bayar** completed her undergraduate education at Selçuk University, Faculty of Agriculture, Department of Plant Protection in 2008 and her postgraduate education at Ege University, Faculty of Agriculture, Department of Plant Protection, Department of Entomology in 2012. She started her PhD. at Ege University, Faculty of Agriculture, Department of Plant Protection, Department of Phytopathology and graduated in 2023. She started her professional life in 2012 at the Aegean Agricultural Research Institute, Department of Plant Protection Department in Izmir and continues to work.

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