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Production of Gibberellic Acid from Maize Tassel and Soybean Waste (Okara) via Solid State Fermentation with *Sacchromyces Cerevisiae*

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ABSTRACT: Gibberellic acid (GA) is a natural plant hormone that functions as a key promoter and regulator of plant growth. Solid-State Fermentation (SSF) provides an economical method for GA production by enabling the recycling of agro-industrial wastes. The aim of this study was to produce gibberellic acid through SSF using maize tassel and okara as substrates, with Saccharomyces cerevisiae serving as the fermenting microorganism. The substrates (maize tassel and okara) were prepared and inoculated with a cultured strain of S. cerevisiae. The effect of an additional supplementary carbon source (jaggery) on GA production was also evaluated. The results demonstrated that the natural nutrient composition of maize tassel and okara sufficiently supported gibberellic acid production without the need for external supplementation. Following fermentation, gibberellic acid was extracted and purified using liquid–liquid extraction followed by Thin Layer Chromatography (TLC). Quantification of the extracted GA was performed using spectrophotometric analysis. Overall, the findings indicated that solid-state fermentation of maize tassel and okara with S. cerevisiae is a viable method for gibberellic acid production. Thus, agricultural by-products despite their environmental challenges hold significant potential as valuable raw materials for the biosynthesis of phytohormones.

KEY WORDS: Maize Tassel; Okara; Gibberellic Acid; SSF; Spectrophotometer; TLC.

I.INTRODUCTION

Gibberellins (GA) are a diverse group of isoprenoid phytohormones that hold a significant importance in agricultural sector. Among the various gibberellins, gibberellic acid (GA_3), is of great industrial and economical importance within a agricultural sector. It is used to increase germination rate of seeds, vegetative growth, stimulate cell division, promote flowering, induce enzyme activity and delay senescence of fruits.

Industrially, GA3 is synthesized through fermentation using the fungus *Gibberellafujikuroi,* alternatively known as *Fusarium fujikuroi*[1]. Some other microorganisms, including yeast can also produce GA_3. Due to its widespread usage, the annual production of GA3 has reached upto 100 tons, with prices ranging from 500 to 2500 rupees/ kg. The main fermentation process for obtaining GA3 is submerged fermentation (SmF). However, the production cost of this process is high owing to low yield and multiple separation steps. In addition, GA3 can also be isolated from plant tissues or produced by chemical synthesis but these processes are complicated and give low yield, and hence not profitable for industrial purposes.

Therefore, solid-state fermentation (SSF) has garnered considerable attention in recent years, primarily due to its economic advantages in producing microbial biomass and metabolites, as well as in valorizing agro-industrial by-products. Furthermore, SSF technology yields high concentration of easily recoverable products with minimal bacterial contamination and reduced waste water generation. Its ability to utilize various low-cost agro-industrial by-products for this transformation is also crucial in the current environmental context [2].

Agricultural by-products constitute some of the most abundant and potentially valuable materials on the planet due to their renewable nature and the fact that they could be used to obtain numerous useful biological and chemical products. Unfortunately, much of the agro-industrial waste produced in India remained unused and was discarded as waste, for

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example.2839.00 kT/year of Maize Tassel and 671.00 kT/year of Soybean Waste (okara) were generated annually as was discarded or burned without any benefit [3].

II.RELATED WORK

Several researchers have reported the production of gibberellic acid using agro-industrial wastes as substrate via different methods. Studies also indicate that *Saccharomyces cerevisiae* can effectively utilize maize and soybean by-products for value-added metabolite production, supporting sustainable and low-cost bioprocess development [4-15].

III. SIGNIFICANCE OF THE SYSTEM

This system provides a low-cost, eco-friendly method for producing gibberellic acid by valorizing agricultural wastes through solid-state fermentation using *Saccharomyces cerevisiae*, promoting sustainable bioprocessing and waste management. The Related works of other scientists are presented in section II, the Methodology is presented in section IV, section V covers the experimental results of the study and discussion, and section VI discusses the future study and conclusion.

IV. METHODOLOGY

Materials
Collection and Preparation of sample

Maize Tassel
Maize Tassel sample was collected from Maize farm in the vicinity of Mhaispur, Tq. Akola Dist. Akola.
The collected maize tassel sample was ground into small particles to facilitate analysis. This was done using a grinder. Collected Maize Tassel sample were ground into small particles and stored in the sterile plastic bag at room temperature for further analysis.

Soybean waste (okara)
3kg of soybeans were collected, washed and then soaked for two days. After two days, the water was drained and the beans were rinsed thoroughly.
Grinding and Filtration: The soaked beans were ground in a grinder with water until achieved a smooth consistency. The ground mixture was filtered through a cheesecloth to separate the liquid (soy milk) from the solid residue (okara). The okara residue was allowed to dry for 24 hours.
Drying and Powdering: Once dried, the okara residue was ground into the fine powder using a grinder. The resulted okara powder is stored in a sterile plastic bag at room temperature for further analysis.

Methods
Sterilization Method:

Table 1.
Protocol used for the sterilization of samples.

No.	sample	Amount	Support	Amount	Autoclave
1	Okara	500gm	Jaggery	250gm	Autoclave at 121^0C for 15 min
2	Okara	500gm	-	-	Autoclave at 121^0C for 15 min
3	Maize	200gm	Jaggery	250gm	Autoclave at 121^0C for 15 min
4	Maize	200gm	-		Autoclave at 121^0C for 15 min

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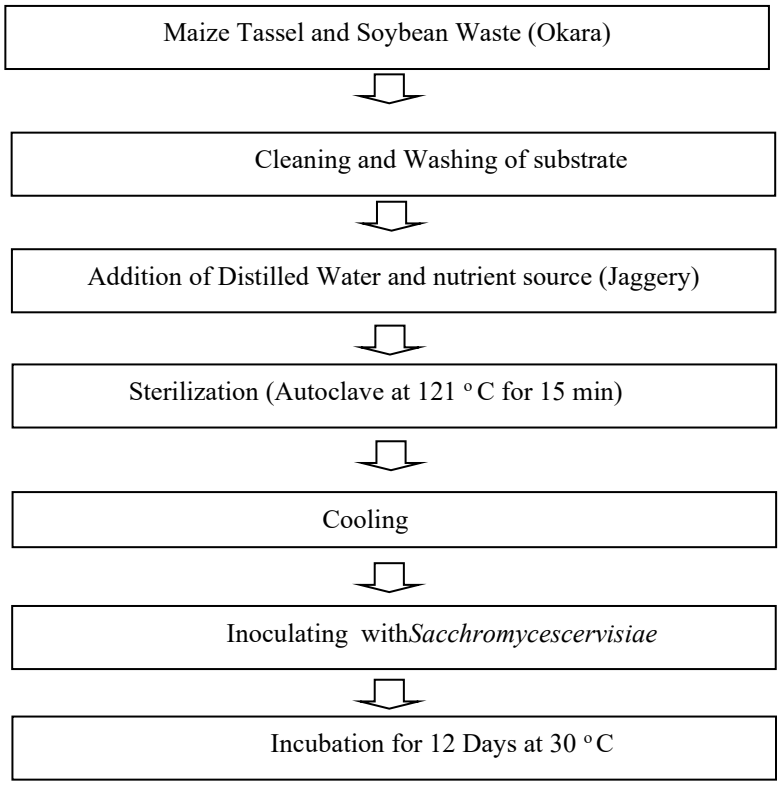
Solid State Fermentation :
Gibberellic Acid was produced by solid State Fermentation using different by product, support and micro-organism.



Experimental Testing Procedures
Determination of Gibberellic Acid by Spectrophotometer:

After 12 days incubation 15ml broth was taken and centrifuged at 8000rpm for 7min to remove biomass. Centrifuged broth was mixed with 2ml zinc acetate and kept for 2 min thereafter, 2 ml potassium ferrocyanide was added and centrifuged at 8000 rpm for 15min. 5ml of supernatant was taken and mixed with 5ml 30% hydrochloric acid and incubated for 75 min. Absorbance was measured at 495nm using standard prepared standard from 10-100ug/ml. (Bhairappa and Nannavare, 2021)

Table 2.

No.	Gibberellic acid(ml)	Phosphomolybdic acid (ml)	D/w	concentration	O.D (780nm)
B	-	15ml	10ml	-	0.00
1	1ml	15ml	9ml	10	0.048
2	2ml	15ml	8ml	20	0.074
3	3ml	15ml	7ml	30	0.120
4	4ml	15ml	6ml	40	0.171
5	5ml	15ml	5ml	50	0.191
6	6ml	15ml	4ml	60	0.289
7	7ml	15ml	3ml	70	0.344
8	8ml	15ml	2ml	80	0.502
9	9ml	15ml	1ml	90	0.546
10	10ml	15ml	-	100	0.591

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Analysis of Gibberellic Acid by thin layer chromatography:

Thin layer Chromatography of extracted Gibberellic Acid was done against standard Gibberellic Acid for quantitative estimation.

The TLC of gibberellic acid extract was carried out on silica gel G coated plates. The extracted organic solvent and standard GA were dissolve in methanol and spotted on TLC plate with the help of glass capillary. They were allowed to dry. These different plates were kept in solvent system containing benzen: n-butanol: acetic acid (6:3:1). The developed spots were visualized under UV after spraying ethanol: sulfuric acid (95:5)

Fig. 1: Development of TLC plate

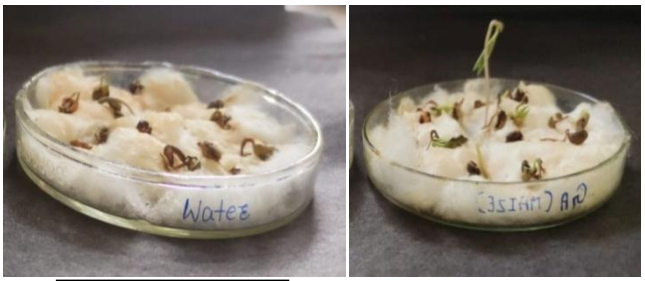
Fig. 2: Spot under UV light

Purification of Gibberellic Acid

After 12 days, culture broth was filtered through whatman No. 42 filter paper. The pH of the filtered broth was adjusted to a range of 2.5 to 3.0 using 0.1N HCl. Ethyl acetate was used as extraction solvent. Culture filtrate was extracted three times using ethyl acetate in separating funnel. Organic layer was allowed to evaporate at room temperature for 2-3 hrs. (Bhairappa and Nannavare, 2021)

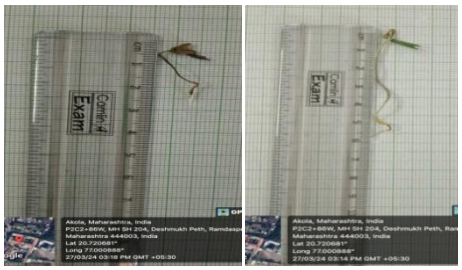
Effect of Gibberellic Acid on Seed Germination of Green Gram.

20 mung bean seeds were added and left them to soak for 5-6 hours. Two Petri dishes were labeled as "control" and "test". Cotton was gently placed in each Petri dish and the cotton was moistened using distilled water. The soaked mung bean seeds were placed in the Petri dishes at equal spacing.



Control Test

Fig 3. Observation of Germination



Control Test

Fig 4. Shoot and Root length measurement

V. EXPERIMENTAL RESULTS AND DISCUSSION

The result of this experiment indicated that the addition of support material to both maize and soybean waste samples enhanced the production of Gibberellic acid when inoculated with *Saccharomyces cerevisiae*. The high concentrations of Gibberellic acid in the supported samples (43ug/ml for Soybean waste with support and 38ug/ml for Maize with support) were compared with the pure samples (10ug/ml for Pure Soybean Waste and 7ug/ml for Pure maize). The concentration was analyzed by spectrophotometer method.

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Determination of Gibberellic acid by Spectrophotometer

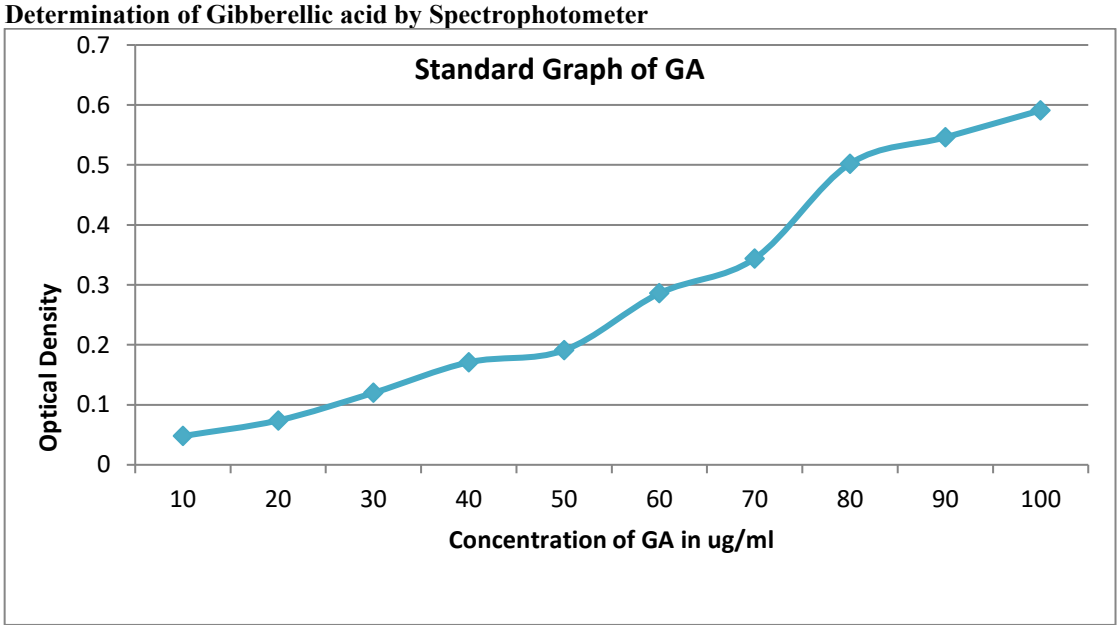

Figure 5. Standard graph of GA

Table 3

Sr. No.	Substrate	Optical density	Concentration in ug/ml
1	Pure Maize Tassel	0.02	07
2	Maize Tassel/support	0.15	38
3	Pure Okara	0.04	10
4	Okara/support	0.17	43

Analysis of Gibberellic Acid by Thin Layer Chromatography:
The gibberellic acid extracted from agro-industrial waste was analysed using thin layer chromatography. The Rf values 0.70, 0.78, 0.72 and 0.75 against the standard Gibberellic acid with Rf value 0.79 confirmed presence of Gibberellins.

Table 4.
The Rf values of respective gibberellic acid are shown in table.

Sr.No.	Name of substrate	Rf (TLC) value
1	Standard Gibberellic Acid	0.79
2	Pure Maize Tassel	0.70
3	Maize Tassel with support	0.78
4	Pure Okara	0.72
5	Okara with support	0.78

Our study revealed that the Rf values of gibberellic acid extracted from both maize tassel and soybean waste are close to the Rf value of the standard gibberellic acid (0.79). This suggests that gibberellic acid is present in both substrates, maize tassel and okara. The minor differences in Rf values could be due to variations in the composition of the extracts.

Effect of Gibberellic Acid on Seed Germination of Green Gram

1st Observation:

Germination Percentage:

$$Germination\% = \frac{Germinated\ seed}{Total\ seed} x\ 100$$

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$Germination\%\ (control) = \frac{13}{15} x\ 100 = 86.66\%$

$Germination\%\ (Test) = \frac{14}{15} x100 = 93.33\%$

It was observed that the germination percentage of test was found to be 6.6% higher than the control.

2nd Observation:

Table 5.

After four days the root and shoot development in both test and control was noted

Shoot/Root (cm)	Control (D/W)	Gibberellic Acid
Shoot (cm)	2 cm	5 cm
Root (cm)	o.5 cm	1 cm

From this table, it was evident that the mung beans treated with gibberellic acid had significant shoot and root development compared to the control group.

Effect of Gibberellic acid on plant growth promoting capacity of Green Gram

Table 6

On day 1

Shoot/leaf length	Control (D/W)	Test-1	Test-2	Test-3	Test-4
Shoot length	10cm	10.5cm	10.5cm	10.8cm	10cm
Leaf length	2.5cm	2cm	2.5cm	2.5cm	2.5cm
New leaf	-	-	-	-	-

After day 6

Shoot length	11cm	11.5cm	11cm	13cm	11cm
Leaf length	3cm	3.4cm	3cm	3.3	3.5
New leaf	-	+	+	+	-

Effect of Gibberellic acid on Plant growth promoting capacity of Maize Plant

Table 7

On day 1

Shoot/leaf length	Control (D/W)	Test-1	Test-2	Test-3	Test-4
Shoot length	4cm	4cm	3.5cm	3cm	4cm
Leaf length	23cm	23cm	18cm	13cm	17cm
New leaf	-	-	-	-	-

After day 6

Shoot length	5cm	5.5cm	5cm	4.5cm	6cm
Leaf length	33cm	24cm	33cm	24cm	38cm
New leaf	+	+	+	+	+

V. CONCLUSION AND FUTURE WORK

From this study, it was concluded that the utilization of maize tassel and soybean waste (Okara) for gibberellic acid (GA) production via solid-state fermentation with Saccharomyces cerevisiae represented a promising approach with significant potential. The study demonstrated the feasibility of converting agricultural by-products into value-added compounds using a sustainable and environment friendly method. The results indicated that the combination of maize tassel and soybean waste provided an effective substrate for supporting the growth of *Saccharomyces cerevisiae* and the biosynthesis of GA. Solid-state fermentation offered several advantages, including low-cost substrates, reduced water consumption, and minimal environmental impact, making it a feasible and profitable option for GA production. Further optimization of fermentation parameters such as substrate composition, moisture content, and inoculum size could enhance GA yield and process efficiency. Additionally, downstream processing techniques should be explored to purify

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and concentrate GA for various applications in agriculture and biotechnology. Overall, this study highlighted the potential of utilizing agricultural residues for GA production through solid-state fermentation, contributing to both sustainable waste management and the development of valuable bioactive compounds.

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