Carbon source utilisation as a basis of variability among different *Ramularia* isolates collected from cotton belt of Odisha

Abstract

The impact of various carbon sources on the growth and sporulation of six different *Ramularia* isolates was investigated by testing eight different sources of carbon in liquid Richard's medium. The mycelial growth was assessed by determining the dry biomass weight, and there were three replicate flasks for each combination of isolates and carbon source. The study showed that the extent of utilization of different carbon sources varied among the isolates, and the highest mean dry biomass weight was recorded by RGKN1 followed by RGGNPR1. Maltose and sucrose were found to be the best source of carbon supporting highest mycelial growth and sporulation. Starch and lactose supported the least mycelial growth and sporulation. The results clearly established the essentiality of carbon source for metabolic activities of the fungus. The study proved the existence of cultural variability among the *Ramularia* isolates basing on their carbon utilisation.

Key Words: carbon sources, biomass, Ramularia isolates, Richard's medium

Introduction

Ramularia is a fungal genus that is responsible for causing leaf spot diseases in a variety of plant species, including cotton. These diseases are a significant threat to crop yield and quality, resulting in significant economic losses for farmers. *Ramularia* leaf spot (RLS) is a devastating disease of cotton, caused by the fungus *Ramulariopsis gossypii* (Speg.) U. Braun (sin. = *Ramularia areola* GF Atk). Its sexual form i.e *Mycosphaerella areola* (Ehrlich & FA Wolf) was reported in Brazil in 2016 (Mehta *et al.*, 2016). Despite the significance of these diseases in causing economic losses, our understanding of the genetic variability among different *Ramularia* isolates and the underlying factors contributing to this variability remains limited. One potential source of variability among *Ramularia* isolates is their ability to utilize different carbon sources (Sghyer & Hess, 2019). Macronutrients, such as carbohydrates, proteins, lipids, and nucleic acids, are composed of essential elements including carbon, hydrogen, nitrogen, sulphur, and phosphorus. These elements play significant roles in mechanisms such as host-pathogen interaction and self-defence mechanisms (Rajderkar, 1966; Walker & White, 2005; Lee et al., 2007). Carbon sources are essential for the growth and survival of fungi, and the ability to utilize a wide range of carbon sources is an important adaptation strategy that allows fungi to survive in diverse environments (Zahra *et al.*, 2011; Gao *et al.*, 2007). Fungi can readily absorb and metabolize a variety of soluble carbohydrates, such as glucose, xylose, sucrose, and fructose. Fungi are also characteristically well equipped to use insoluble carbohydrates such as starches, cellulose, and hemicelluloses, as well as very complex hydrocarbons such as lignin. Many fungi can also use proteins as a source of carbon and nitrogen (Britannica). Carbon source utilization patterns may reflect differences in the metabolic pathways and nutrient requirements of different fungal isolates, which may in turn contribute to differences in their pathogenicity and virulence (Brown, 2023). The results by Gao *et.al*, 2007 indicated that the influence of carbon concentration and C:N ratio on fungal growth and sporulation is strain dependent. The cotton belt of Odisha is a significant cotton-producing region in India, where *Ramularia* leaf spot disease has been a major concern lately

for cotton farmers. In this research paper, we investigated the carbon source utilization patterns of a collection of *Ramularia* isolates from the cotton belt of Odisha. Our objective was to explore the potential role of carbon source utilization as a basis for genetic variability among the isolates and its implications for understanding the pathogenicity and virulence of this devouring fungal genus. To achieve our objective, *Ramularia* isolates were collected from cotton plants in different locations across the cotton belt of Odisha. They were cultured on media containing different carbon sources, such as glucose, fructose, sucrose, and maltose, and their dry biomass weight and sporulation were measured. Our research findings will contribute to a better understanding of the genetic variability and adaptation strategies of *Ramularia* isolates in the cotton belt of Odisha. This knowledge will be valuable for developing effective strategies to control this disease and improve cotton yield and quality in this important cotton-producing region.

Materials and Methods

To investigate the impact of various carbon sources on the growth and sporulation of six different *Ramulariopsis* isolates (RGBP1, RGGNPR1, RGBLGR1, RGNP1, RGKN1, RGKG1) a study was conducted by testing eight different sources of carbon. The fungus's utilization of these sources was analysed in liquid Richard's medium. The C/N ratio was kept constant, and the quantities of carbon compounds were adjusted to provide the same amount of carbon as present in 50.00 g of sucrose, in a litre of Richard's medium based on their molecular weight (Table-1). A basal medium devoid of any carbon source was used as the control. After inoculating 2 ml mycelial suspension into 75 ml liquid medium in 100 ml Erlenmeyer flasks, they were incubated for 25 days at 19 ± 2°C temperature. Mycelial growth was assessed by determining the dry biomass weight, and there were three replicate flasks for each combination of isolates and carbon source (Itoo and Reshi, 2014). The mycelial biomass of each treatment combination were harvested, filtered, and dried in an oven at 60°C, and the fungal dry weights were recorded. The average mycelial dry weight was calculated and compared to observe the variability in growth among the isolates.

Table 1- Different carbon sources along with their amount to be used in basal medium.

Carbon Source	Molecular Formulae	Molecular weight (g/mol)	% Carbon	Weight(g) per litre of medium
Lactose	$C_{12}H_{22}0_{11}$	342.30	42.11	50.00
Maltose	$C_{12}H_{22}0_{11}$	342.30	42.11	50.00
Sucrose	$C_{12}H_{22}0_{11}$	342.30	42.11	50.00
Glucose	$C_6H_{12}0_6$	180.16	40.00	52.63
Fructose	$C_6H_{12}0_6$	180.16	40.00	52.63
D-Galactose	$C_6H_{12}0_6$	180.16	40.00	52.63
Starch	$C_6H_{10}0_5$	162.16	51.81	47.36
D Sorbitol	$C_6H_{14}0_6$	182.17	39.56	52.63

Results and Discussion

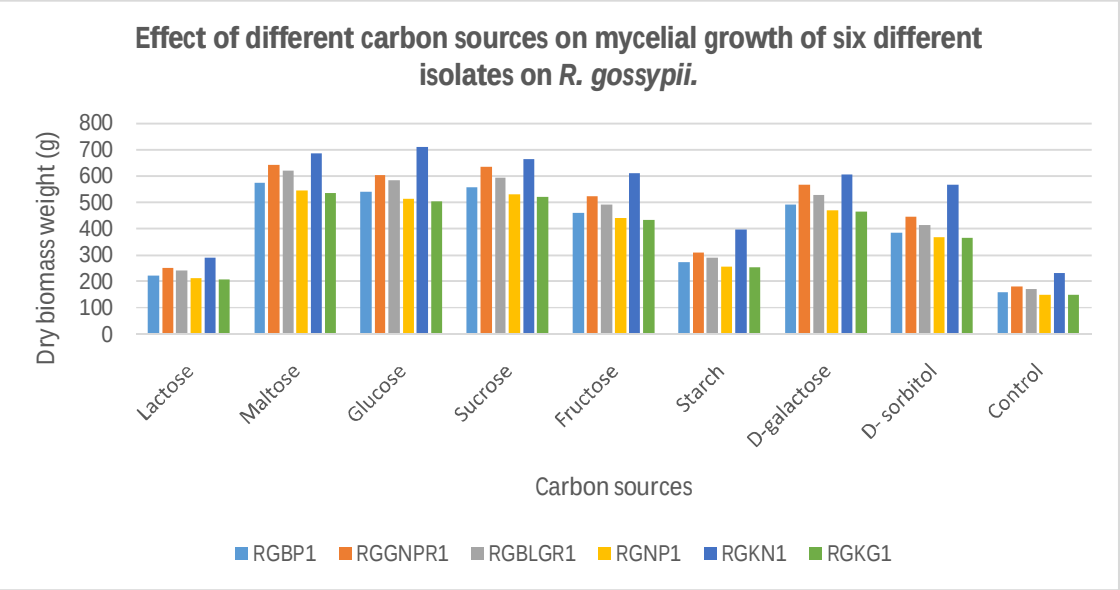
The study was conducted in liquid conditions. The fungus utilized all the carbon sources tested, but the extent of utilization differed with the type of carbon sources. The fungal growth was measured after 25 days of incubation at 19 ± 2°C temperature. There was an overall observation of variability among the tested isolates basing on the way they utilise

different carbon sources. From the present investigation it was noticed that the highest mean dry biomass weight was recorded by RGKN1 (529.37 mg) followed by RGGNPR1 (462.72 mg). This observation helped to come onto a conclusion that both these isolates utilised the sources in the most efficient manner among the rest and yielded best growth and sporulation. The next best isolates taking into consideration carbon utilisation, were found to be RGBLGR1 (437.24 mg) and RGBP1 (407.28 mg). Isolates RGNP1 (387.31mg) and RGKG1 (382.10 mg) recorded more or less similar carbon utilisation efficiency with no significant variability. In liquid condition, dry weight of mycelium and sporulation were recorded and are presented in Fig 1 and Table-2. Among all the carbon sources, maltose was found to be the best source of carbon as it recorded maximum dry biomass weight (601.08 mg) followed by sucrose (583.77 mg) , glucose (575.43 mg) and D-galactose (521.94 mg). While, other carbon sources viz. fructose (493.46 mg) and D-sorbitol (424.87 mg) supported moderate fungal growth. Minimum dry biomass weight was observed in starch (296.94 mg) and lactose (237.18 mg) among different carbon sources. Similarly, abundant sporulation was also observed in maltose and sucrose, whereas glucose and fructose supported good sporulation. D-galactose and D-sorbitol supported moderate sporulation of the fungus. There was very little growth and sporulation recorded in starch and lactose containing media followed by the least in the medium devoid of any carbon source (control). It clearly established the essentiality of carbon source for metabolic activities of the fungus from the present investigation. It can be concluded from the present study that maltose and sucrose were the best carbon sources, whereas glucose, D galactose and fructose were next best in respect of their merit in both growth and sporulation of *R. gossypii*. Our results very much coincided with the results of Dasarathabhai (2005) who conducted an experiment to determine the optimal carbon sources for growth and sporulation of ***R. gossypii*** where sucrose was found to be the best carbon source, recording the maximum dry mycelial weight followed by glucose and galactose. Goyal in 1977 and Ayed *et al*. (2020) identified maltose as the preferred carbon source for fungus which was also found by us as the most promising carbon source in this experiment.

Table 2 - Effect of different carbon sources on sporulation of six different isolates on *R. gossypii.*

Carbon source	RGBP1	RGGNPR1	RGBLGR1	RGNP1	RGKN1	RGKG1
Lactose	++	++	++	+	++	+
Maltose	++++	++++	++++	++++	++++	+++
Glucose	+++	++++	++++	+++	++++	+++
Sucrose	++++	++++	++++	+++	++++	+++
Fructose	+++	++++	+++	+++	++++	+++
Starch	++	++	++	++	+++	++
D-galactose	++	+++	+++	+++	+++	++
D- sorbitol	+++	+++	++	+++	+++	++
Control	++	+	+	+	++	+

Fig 1- Effect of different carbon sources on dry biomass weight of six different isolates on *R. gossypii.*



Conclusion

To sum up, this study has shed light on the utilization of different carbon sources by *R. gossypii*, highlighting the variability among the tested isolates and the importance of carbon sources for fungal growth and sporulation. The findings suggest that maltose and sucrose are the most promising carbon sources for the fungus. These results may be useful in optimizing the culture conditions for *R. gossypii* and improving its further study. However, there is still much to be explored regarding the variability among different *R. gossypii* isolates. Future studies could investigate the genetic and metabolic factors that contribute to the observed differences. Additionally, it would be interesting to explore the impact of other environmental factors, such as temperature, pH, and nutrient availability, on the growth and metabolism of *R. gossypii*. Such studies could help us gain a deeper understanding of the physiology of this fungus and its mechanism of causing grey mildew disease in cotton plants.

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