IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

PHYSICAL MAPPING OF DIFFERENT MORPHOLOGICAL CHARACTERS IN *NEUROSPORA CRASSA*

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Abstract

Neurospora crassa is a type of red bread mold of the phylum ascomycota meaning “nerve spore” in Greek, refers to the characteristic striation on the spores. *Neurospora crassa* is use as a model organism because it is easy to grow and passed a haploid life cycle that makes genetic analysis of genetic recombinations is facilitate by the ordered arrangement of the products of meiosis in Neurospora ascospores. Orderly arrangement of tetrad is used in this present piece of work to know the position of different morphological characters and also used for physical mapping of genes such as spore color, sporangium size, etc.

Keywords: Tetrad, morphological character, physical mapping

Major Findings

Neurospora may be used as model organisms for physical mapping of different genes and the strategy may be used for similar organisms. The order of tetrad may be assessed for physical mapping. In the present study the characters such as color colony, size of sporangium, size of spore, septa or asepta, length of sporangium, color of spore and shape of sporangium with the help of recombinant frequency between the locus and centromere.

Key Points: Neurospora is the filamentous fungus Neurospora crassa possesses a number of distinct genome-defense mechanisms and has been crucial in the advancement of contemporary genetics. *Recombinant frequency* is the frequency at which a single chromosomal crossing happens between two genes during meiosis. A chromosome's *centromere* is a restricted region that is essential for aiding in the cell's DNA division during mitosis and meiosis. It is, specifically, the area where the spindle fibers of the cell are attached.

Introduction

Neurospora is a well-known model organism to study the gene map based on linkage associated with different phenotypic characters (Honda. Et. Al. 2020). In the present study the Neurospora was isolated from the soil and identified by using Gilman's and Barnnet manual (Chandani and Sushma 2021). The various characters such as spore size, spore color, size of sporangium, length of sporangium, shape of sporangium has been studied, about twenty different cultures with various replicates (n =7) has been used to calculate the cross over value between the centromere and a particular phenotypic character. On the basis of cross over value linkage map has been created which shows the apparent physical position of associated genes on chromosome (Copeland, N. G., & Jenkins, N. A. 1991). The study was a type of fundamental research which leads to the better understanding between the frequency of a phenotype and their genotypic locations(Copeland, N. G., & Jenkins, N. A. 1991).

Material and Method

Isolation and purification of *Neurospora* species from soil sample

Soil sample was collected with the help of borer and sealed in zipper bags from P.G. Department of Biotechnology, Bhagalpur field. Five test tube were taken and labeled it 10^{-1} to 10^{-5} , then Serial Dilution Method(Blodgett, R. J. (2009) was performed. Then after Vogel's N Media (Atlas, R. M. 2010) prepare and Poure Plate (Terrones-Fernandez. Et. Al. 2023) method was performed.

Table1.0: Vogel's N Media

Composition in liter	Amount
Sucrose	15.0g
KH_2PO_4	5.0g
Trisodium citrate.$2H_2O$	3.0g
NH_4NO_3	2.0g
$MgSO_4.7H_2O$	0.2g
$Cacl_2.H_2O$	20.0ml
Biotin Solution	5.0ml
Trace element solution	5.0ml

Table2.0: Trace element solution

Composition per 100ml	Amount
Citric acid.H_2O	5.0g
$ZnSO_4$	5.0g
$Fe(NH_4)_2(SO_4)_2.6H_2O$	1.0g
$CuSO_4.5H_2O$	0.25g
H_3BO_3.anhydrous	0.05g
$MnSO_4.H_2O$	0.05g
$Na_2MoO_4.2H_2O$	0.05g

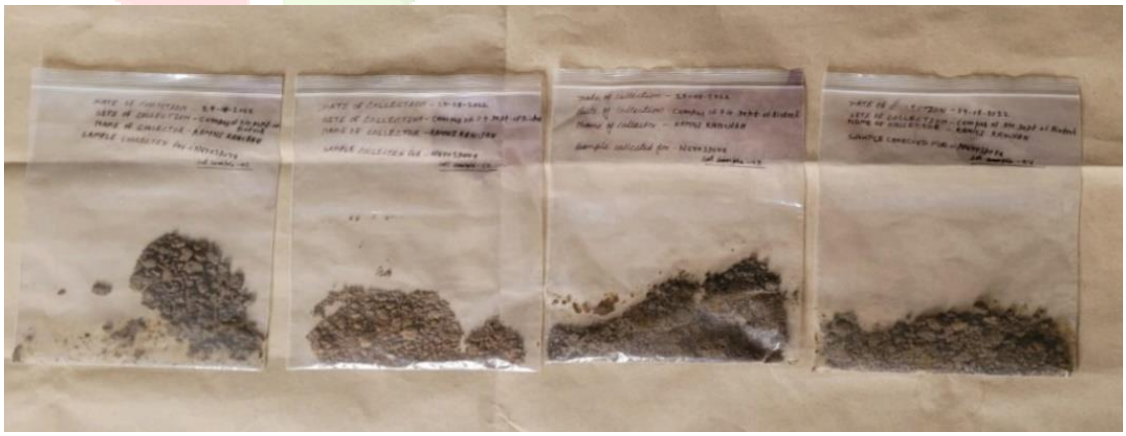


Fig1.a: Soil Sample collected from the field of P.G. Dept. of Biotechnology TMBU Bhagalpur

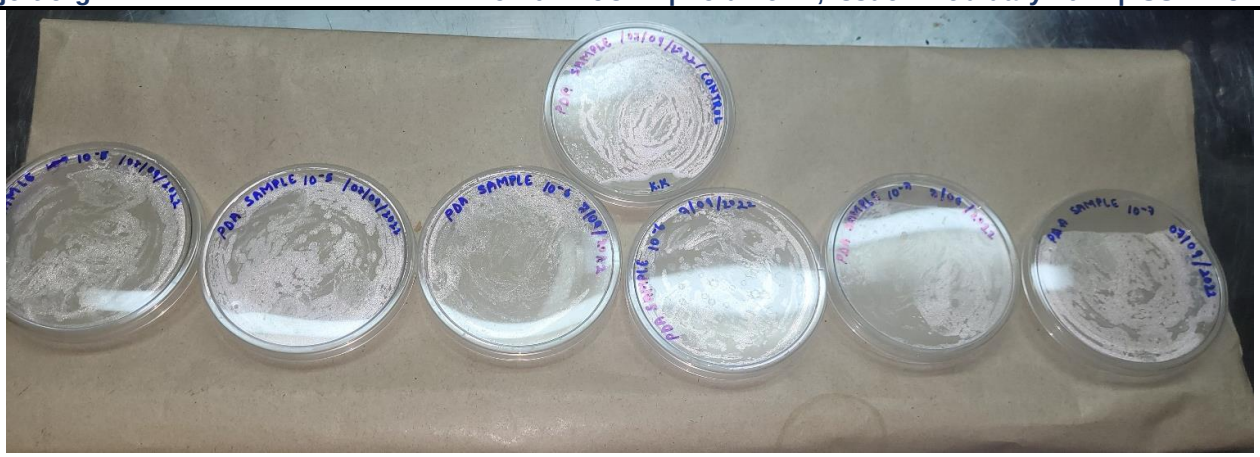


Fig. 1.b: Pore Plate of Soil Sample

Preparation of Pure Culture

To prepare the isolate of pure culture slant culture (Dr Solunke, A. B. 2019) was done. Twenty culture tube was prepared.

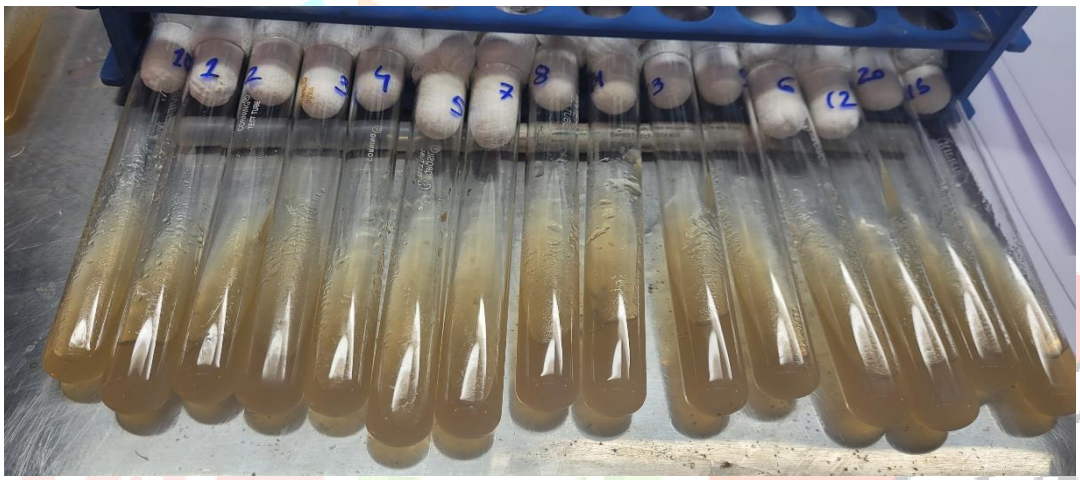


Fig2.0: Slant culture of *N. crassa*

Morphological characteristics of isolated Neurospora microscope

The unknown culture isolate was identified by the help of Lactophenol Cotton Blue (LPCB) (Leck, A. (1999) to observed the morphological characteristics by the help of Gilman's and Barnnet method (Chandani and Sushma 2021) such as hyphae, conidial head and arrangements under the microscope.

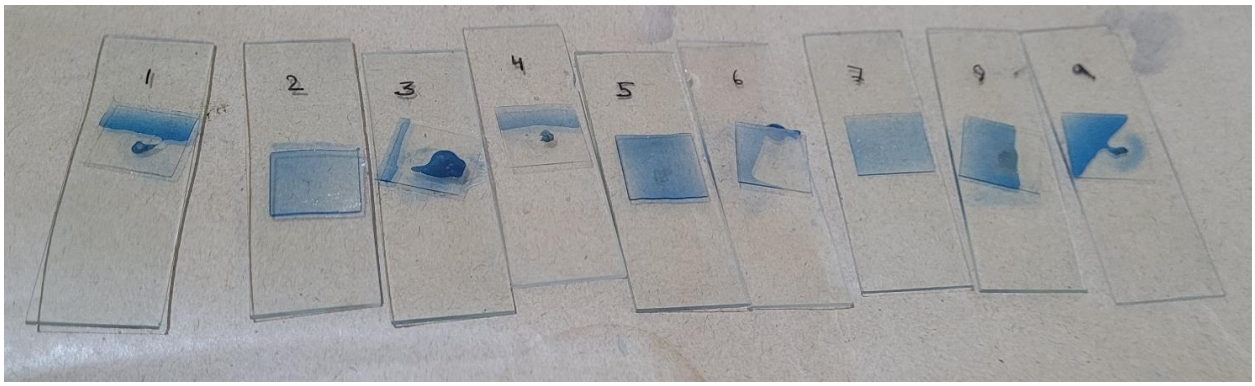


Fig. 3.0: Slides for detection of morphology of fungi

Assessment of Morphological Character

Fungal taxonomy was previously determined by phenotypic characteristics that could be observed. To identify fungi, morphological analysis, microscopy, photography, and specimen descriptions are crucial. Thus, staining, mounting, and slide preparation methods are described (Senanayake et. Al. 2020). To study the microscopic examination of culture different characters was studied like color of colony, septa, shape of spore, color of spores, size of sporangia, size of spore, length of sporangium, and shape of sporangium are viewed under the photograph enabled compound microscope.

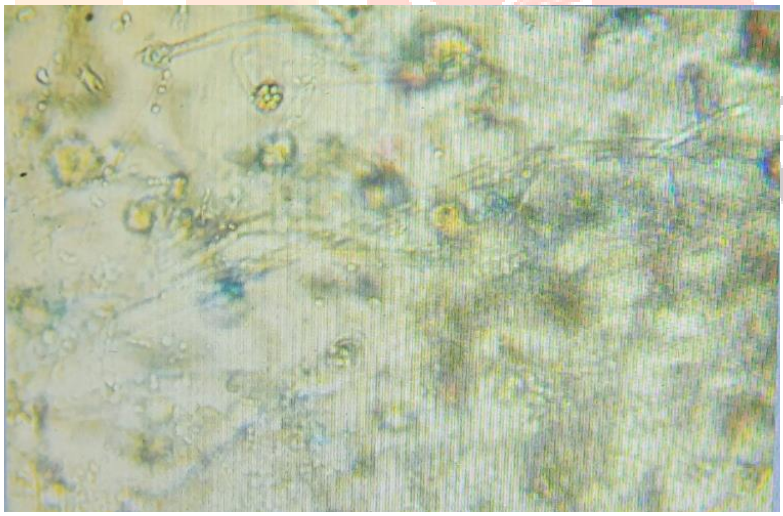


Fig 4.0: Microscopic visuals of isolates

Preparation of linkage map

In each arm of *Neurospora crassa*, the frequency of crossing-over in the two short areas on opposite arms, next to the centromere of the mating-type chromosome, is determined separately by at least two genes that have an additive and equal effect. The probability of recombination and second-division segregation of loci located within these regions varies greatly due to the segregation caused by these genes during inbreeding. The recombination frequency about the centromere alone may be the cause of the relative increase or reduction in their centromere distances; recombination values between loci located outside of these sensitive regions are unaffected (Rifaat, O. M. 1969).

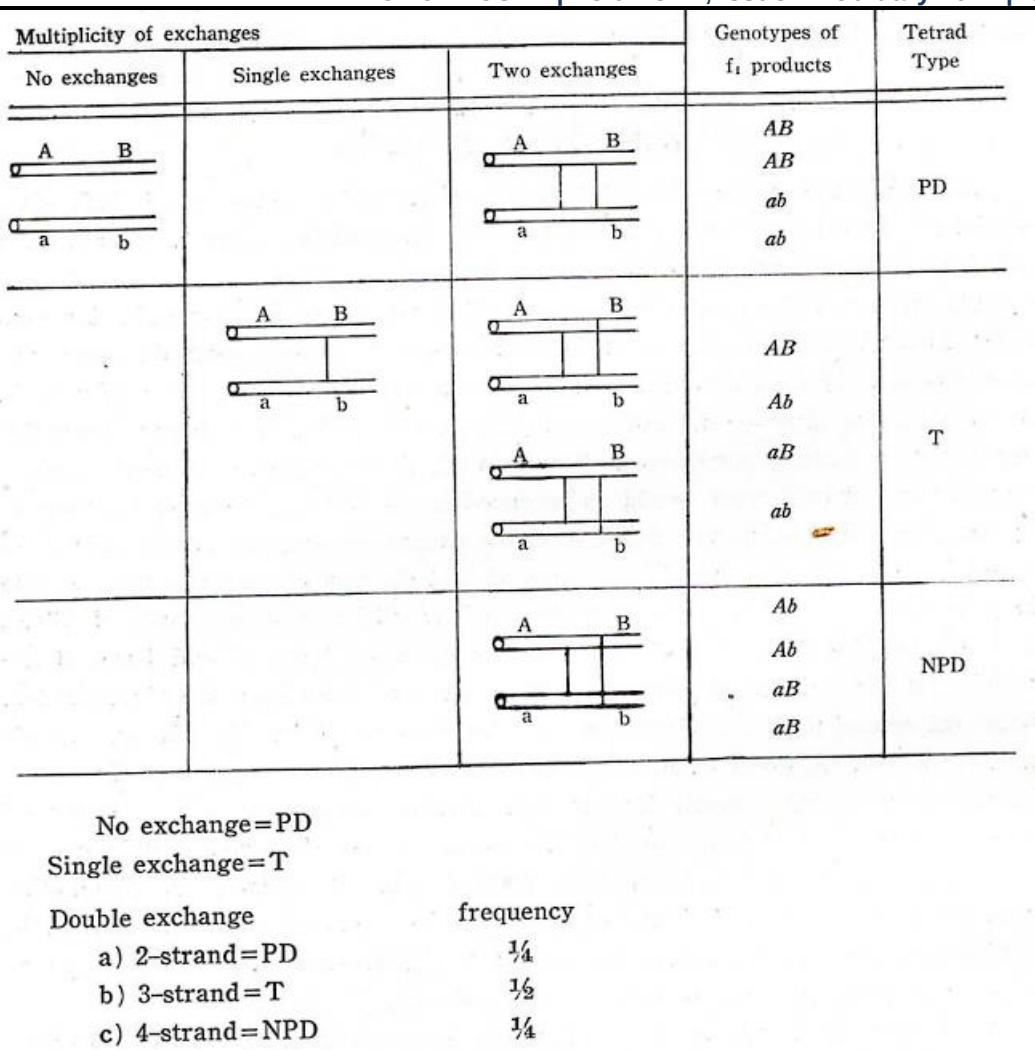
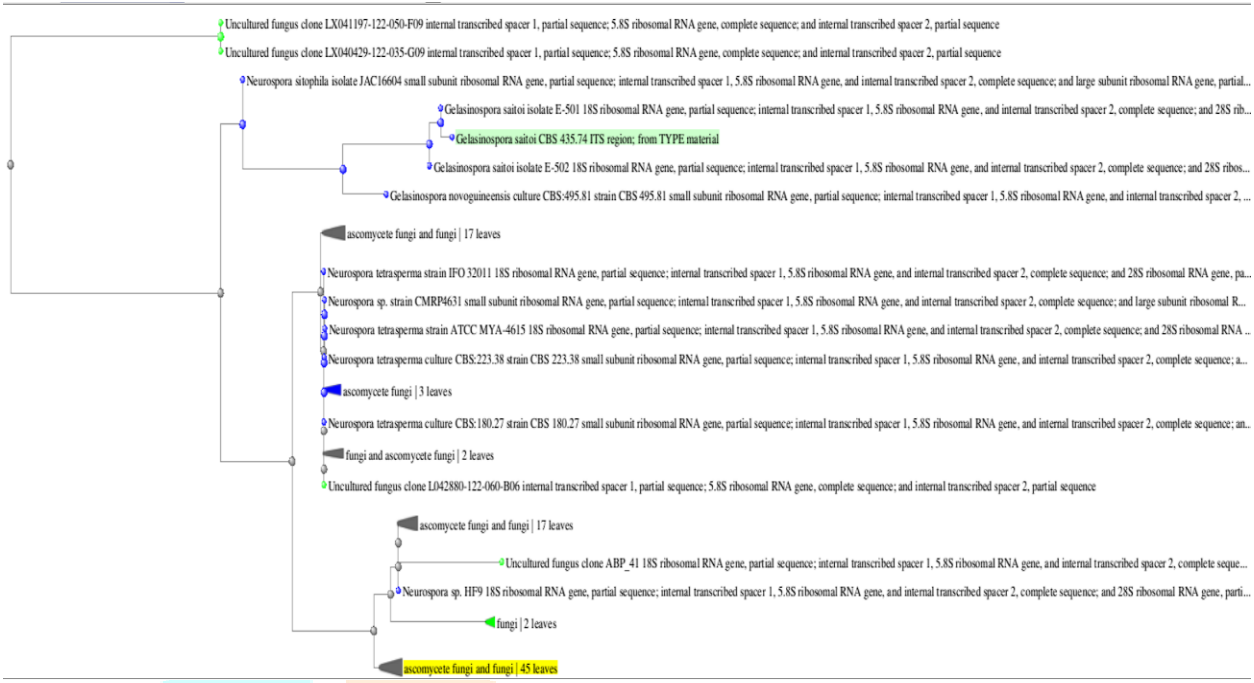


Fig 5.0: Tetrad analysis (C. Shields Gowans)

Phylogenetic relationship of studied in Neurospora

A bioinformatics tool called NCBI aids in the creation of phylogenetic trees. Open the appropriate browser and navigate to the NCBI's official website at (https://www.ncbi.nlm.nih.gov/). Next, type "neurospora" into the search box, choose "nucleotide," and press the "Search" button. The relevant link will then open, leading to the opening of a new page with the specifics of the relevant options. To proceed, select the Run BLAST option. Once more, a new page opened that displayed the BLAST page. Clicking the BLAST option resulted in the appearance of a new webpage followed by the result page. The phylogenetic tree will then show up when you select the distance tree option.

Fig 6.0: Phylogenetic analysis of *N. crassa* [13 – Ncbi link]



Result

Isolation and Purification of Neurospora from soil

The soil sample was taken and packed or sealed under the plastic bags as shown in the figure 8.0. Then after place into freezer for keeping it safe.

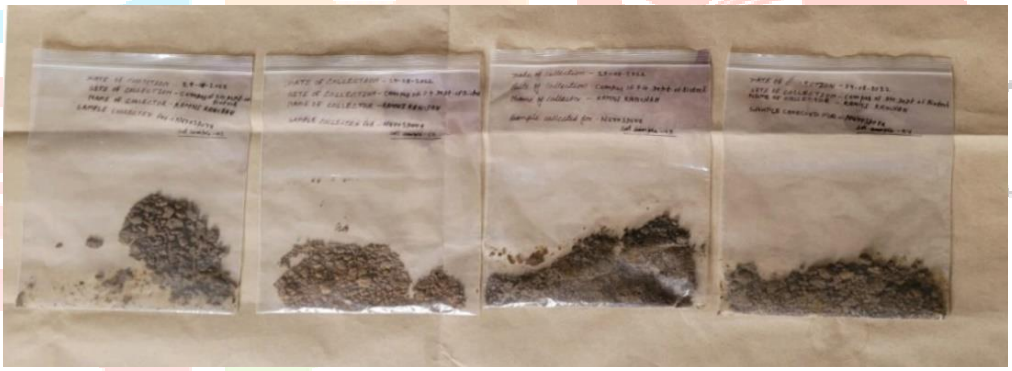


Fig 7.0: Soil sample

Purification of pure culture

In each test tube the cottony like fungi was appeared in each slants.

Morphological characterization of isolated Neurospora

By the help of Gilman's and Barnett manual [2] the identification of the unknown isolates of fungi was performed and viewed under the photographic enabled microscope.

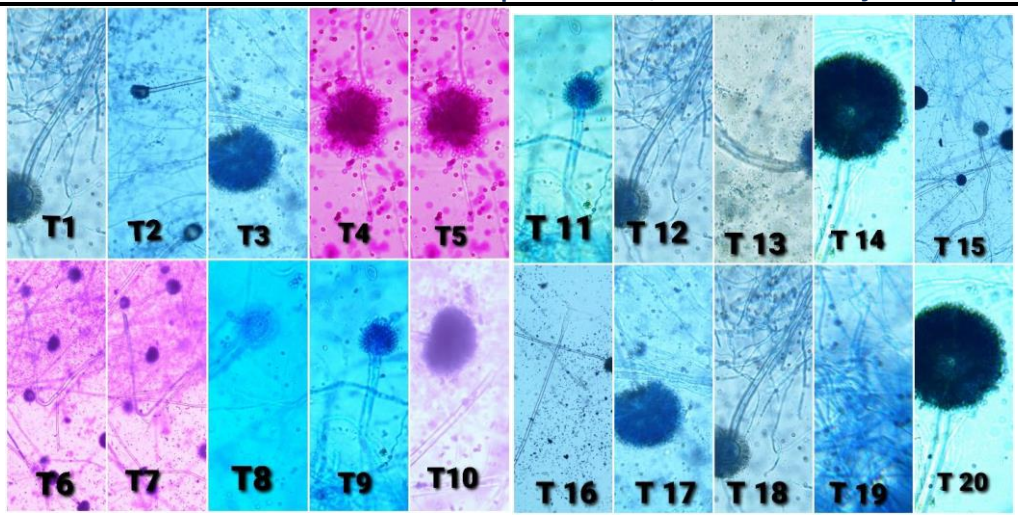


Fig 8.0: Microscopic slides of various isolates

Assessment of Morphological Characters

Table 3: Identification of different types of morphological characters

Test Tube	Colony color	Size of Sporangium (mm)	Size of spore (mm)	Septa	Length of sporangium (mm)	Color of spore	Shape of sporangium
T1	Light grey	37.73	6.3	+	168.96	Grey	Globular/ Spherical
T2	Whitish	19.04	3.32	+	374.4	Grey	Dumble
T3	Whitish	35.9	95.9	+	93.61	White	Spherical
T4	Whitish	23.76	6.61	+	105.6	White	Spherical
T5	Whitish	19.8	5.3	-	132	Grey	Spherical
T6	Grey	26.45	5.29	-	88.88	White	Spherical
T7	Grey	21.16	7.14	+	192.72	Grey	Spherical
T8	Whitish	17.68	6.3	-	150.06	Grey	Spherical
T9	Whitish	36.96	9.24	-	475.2	Grey	Spherical
T10	Whitish	20.21	8.76	+	390.72	White	Spherical
T11	Grey	29.04	9.5	+	114.84	White	Spherical
T12	White	52.8	7.9	+	25.8	White	Spherical
T13	Grey	35.3	8.9	-	467.2	White	Spherical
T14	White	27.3	5.9	+	94.6	White	Spherical
T15	White	20.1	7.4	-	190.3	Grey	Spherical
T16	Grey	18.23	6.8	-	155.2	White	Spherical
T17	White	22.6	5.9	+	104.3	Grey	Spherical
T18	Yellowish grey	36.7	8.9	-	97.4	White	Spherical
T19	Yellowish grey	27.9	6.8	+	90.5	Grey	Spherical
T20	White	21.2	17.02	-	149.3	White	Spherical

Colony Color – The various types of colony color of *N. crassa* is observed in the slants and the following types of colonies were observed are as follow:

1. Light Grey – Out of twenty colonies only single colonies was appeared as light grey that is T1 test tube.
2. White – Out of twenty colonies five colonies was white in color named as T12, T14, T15, T17 and T20 test tubes.
3. Whitish – Out of twenty colonies seven colonies has whitish appeared named as T2, T3, T4, T5, T8, T9 and T10 test tubes respectively.
4. Grey – Out of twenty colonies five colonies was grey appearance named as T6, T7, T11, T13, and T16 test tubes.
5. Yellowish Grey – Out of twenty colonies two colonies was yellowish grey in color T18 and T19 test tubes.

Size of Sporangium – The size of sporangium of *N. crassa* ranges from 19.04mm to 37.73mm.
Size of Spore – The size is varying from 3.32 mm of T2 to 95.9 mm of T3 test tubes respectively
Color of Spores – By observing the color of spore the grey and white color was appeared. Out of twenty test tubes T1, T2, T5, T7, T8, T9, T15, T17, and T19 test tubes are of grey color and T3, T4, T6, T10, T11, T12, T13, T14, T16, T18, and T20 test tubes are of white color.
Septa – Out of twenty colonies eleven colonies (T1, T2, T3, T4, T7, T10, T11, T12, T14, T17 and T19) was septa and nine colonies (T5, T6, T8, T9, T13, T15, T16, T18 and T20) were aseptate of *N. crassa.*
Shape of sporangium – Only single colonies that is T2 test tube was dumble in shape and rest colonies was spherical in shape.
Preparation of linkage map
To study the mapping in the *N. Crassa* the characters was distinguish between groups.

Table 4: Study of different characters of *N. crassa*

Study of frequency of character	**Color of Colony (Neurospora)**	Light Grey	1
		Whitish	5
		Grey	5
		Yellowish Grey	2
		White	7
	Size of Sporangium	Group 1 (11-20mm)	6
		Group 2 (21-30mm)	8
		Group 3 (31-40mm)	5
		Group 4 (41-50mm)	1
	Size of Spore	Group 1 (3-5mm)	1
		Group 2 (6-8mm)	12
		Group 3 (9-11mm)	6
		Group 4 (11-13mm)	1
	Septa	Positive	11
		Negative	9
	Length of Sporangium	Group 1 (80-180mm)	13
		Group 2 (181-280mm)	5
		Group 3 (281-380mm)	1

		Group 4 (381-480mm)	2
	Color of Spore	Grey	10
		White	10
	Shape of Sporangium	Spherical	18
		Globular	1
		Dumble	1

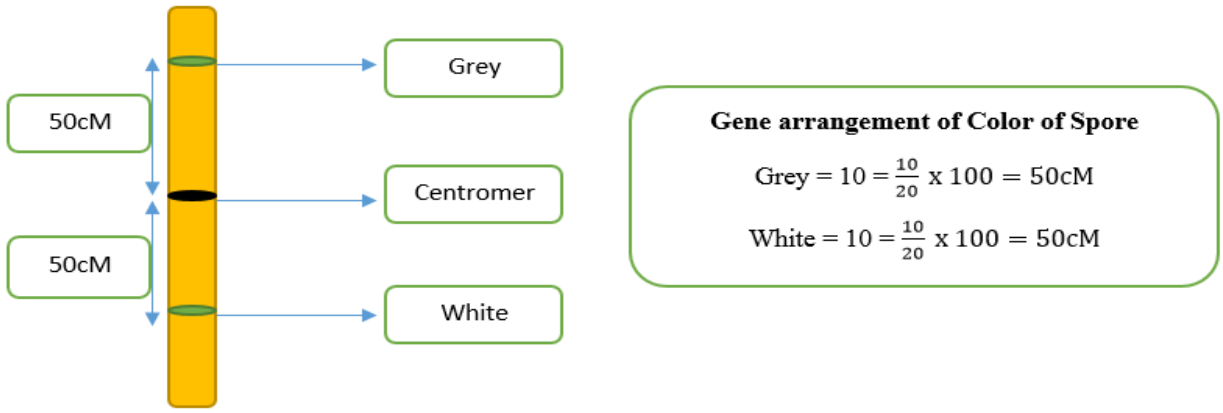


Fig 9.a: Chromosomal arrangement of Spore color *N. crassa*

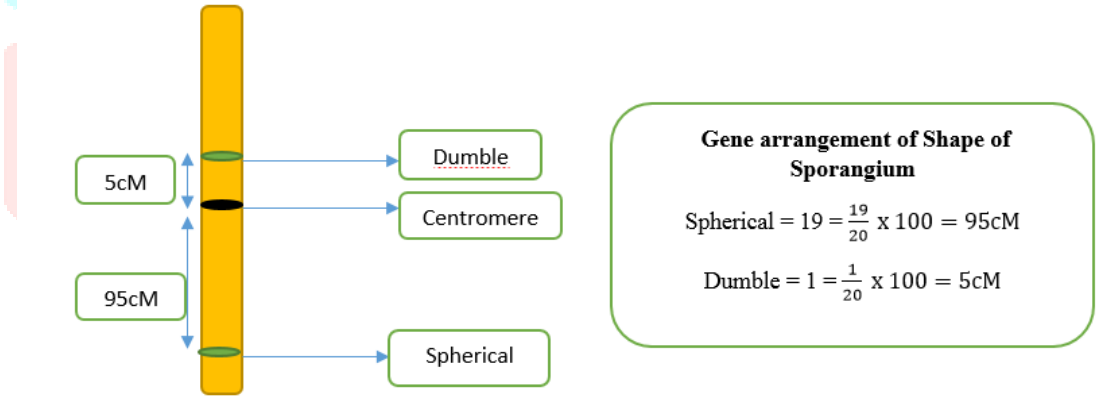


Fig 9.b: Chromosomal arrangement of shape of sporangium *N. crassa*

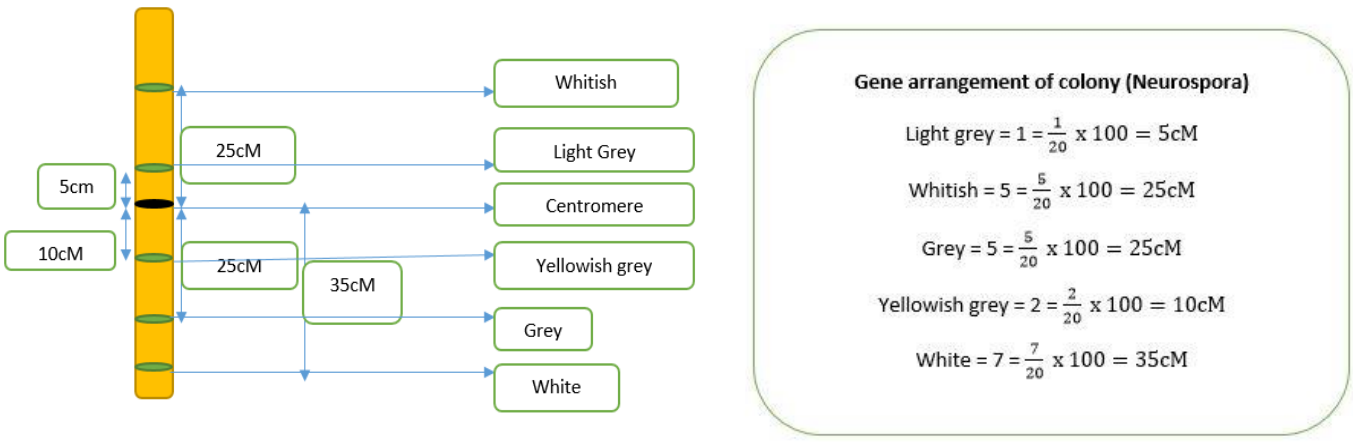


Fig 9.c: Chromosomal arrangements of colony color *N. crassa*

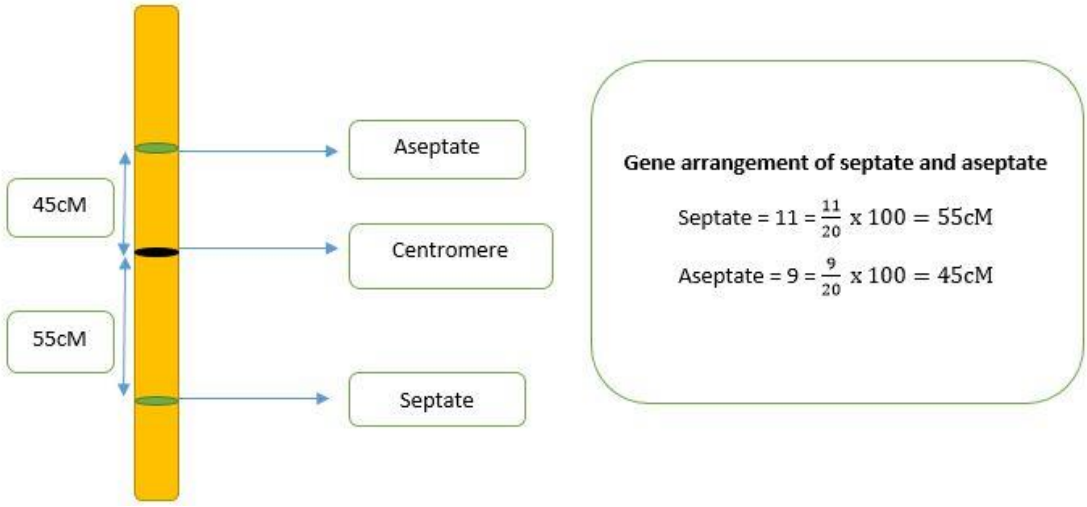


Fig 9.d: Chromosomal arrangements of septate and aseptate gene *N. crassa*

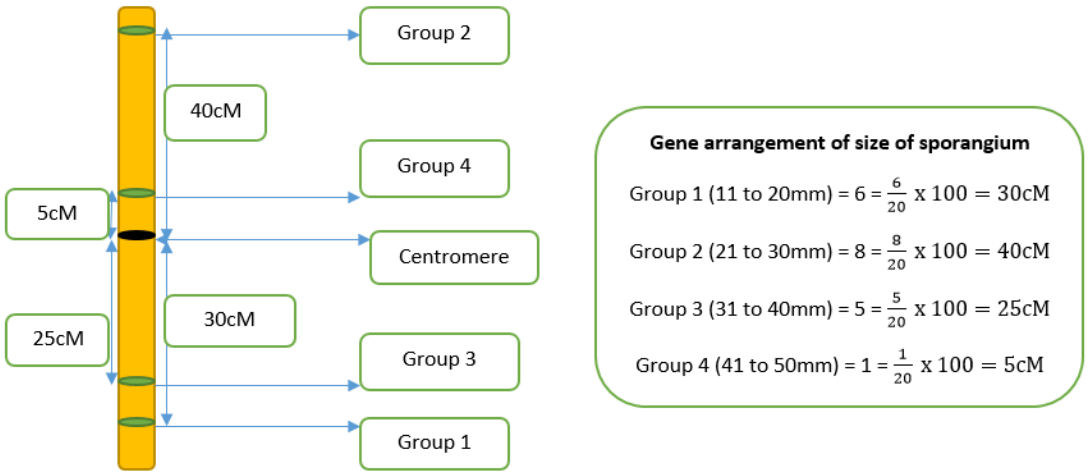


Fig 9.e: Chromosomal arrangement of size of sporangium *N. crassa*

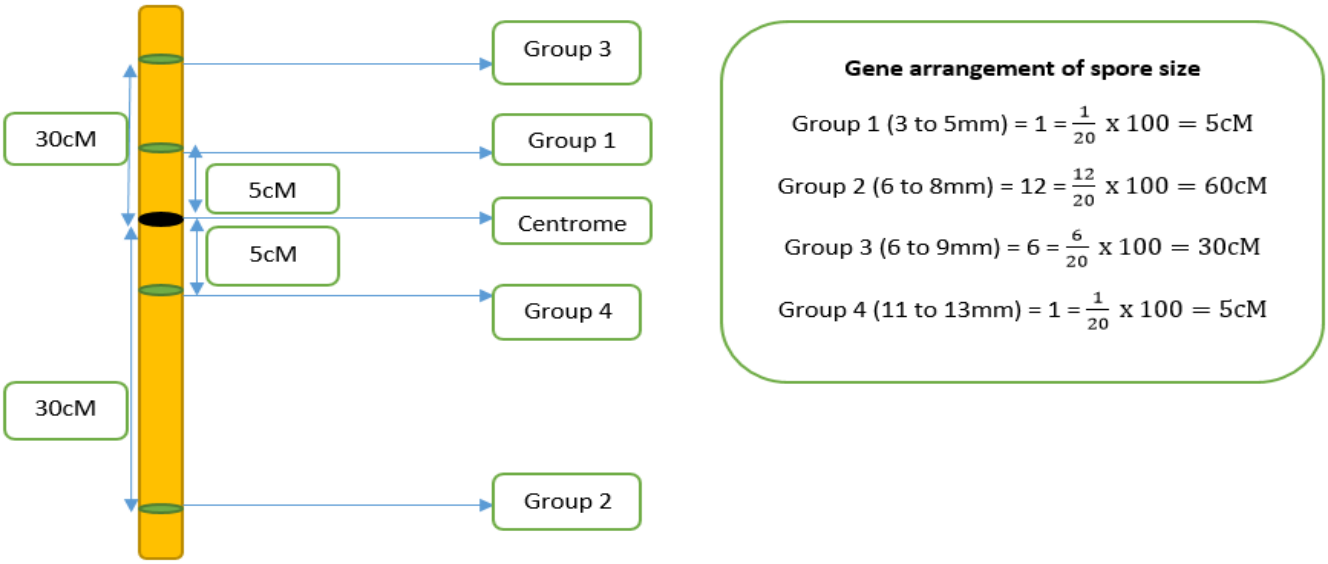


Fig 9.f: Chromosomal arrangement of spore size of *N. crassa*

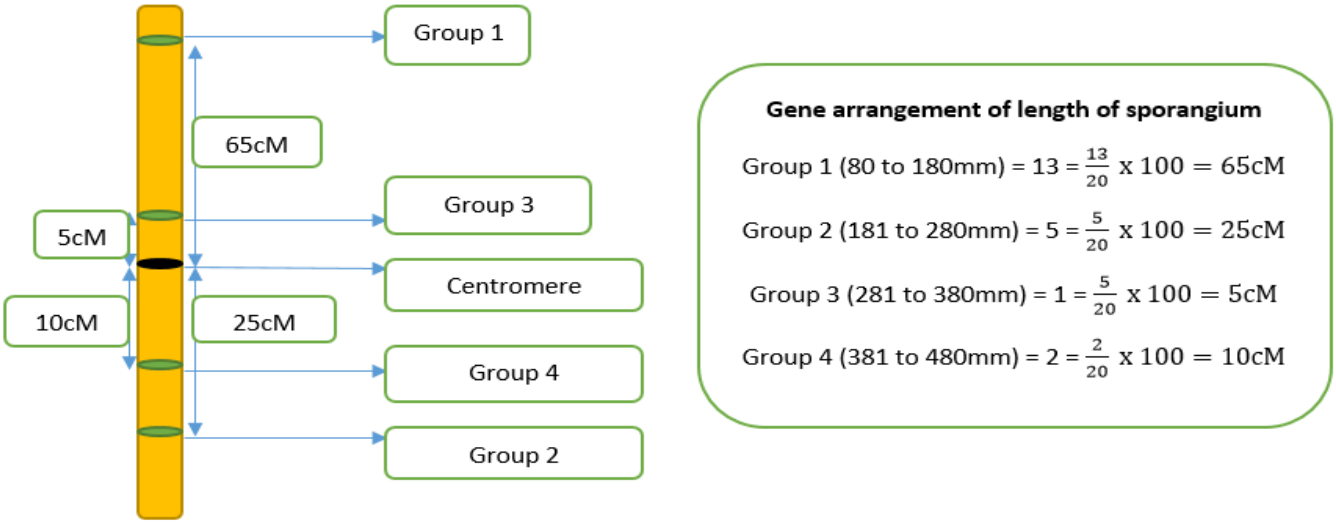


Fig 9.g: Chromosomal arrangement of length of sporangium in *N. crassa*

Data availability statement

The data generated in the manuscript is the original experimental data, compiled in tabular and graphical forms. The data is not used for any other publication, dissertation and thesis work**.**

Discussion

Neurospora crassa is used as a model organism because it is easy to grow and has a haploid life cycle that makes genetic analysis simple since recessive traits will show up in the offspring. Analysis of genetic recombination is facilitated by the ordered arrangement of the products of meiosis in *Neurospora*. The colony colour is varying in a range of, grey to light grey, yellowish grey and yellow grey and white. Similarly, the other morphological characters showed different traits and the frequency of each trait is calculated for their position with respect to the traits of other character. This results in a relative positioning of different characters on chromosomes. Therefore, the different morphological characters can be studied for their relative physical position on different chromosomes through calculating the recombination frequencies between different concerned characters.

Author's Contributions

The authors declare that there is no conflict of interest among them. **KK** does all the experimentation as a part of their dissertation work. **VA** helps in the paper writing and data compilation. **RKV** is the supervisor and supervise the whole work of experimentation, manuscript editing and data verification.

Conflict of Interest

There is no conflict of interest between authors.

Ethical Statement

Although it is impossible to prove the negative assertion that Neurospora is harmless. But it is not essential to obtain ethical statement to work upon *Neurospora.*

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