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Morphological Characterization of *Rhizoctonia* species and *Cephalosporium* species on Cassava *(Manihot esculenta (L.))*

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Abstract
Cassava is a very good source of food security in the southern part of ethiopia despite tremendous inflictions due to bacterial wilt caused by rhizoctonia spp and cephalosporium spp. This research was initiated to characterize the colonial morphology of rhizoctonia spp and cephalosporium spp under laboratory conditions. Completely randomized design had been employed in which the treatments were replicated thrice. The result indicated that these selected fungal genera (rhizoctonia spp and cephalosporium spp) had shown variation in morphological characterization. Besides it exhibited organ specificity in the development of diseases symptoms; which need further studies to answer the question why. Morphologically, rhizoctonia spp. was septated, repeatedly branched, thick, form buds and hyphae are interlocked. Asexua fruit bodies and spores lacking. The incitants of bacterial wilt diseases are too diverse. Thus, more researches are needed in production, post-harvest technology, processing, marketing and determination of quality. More fungal characterization and identification researches should be done on fungal diseases of cassava.

Key words: *- Cephalosporium spp., culture media, morphological characterization, rhizoctonia spp.,*

Introduction

Cassava (*Manihot esculenta* Crantz) is increasingly crucial in the southern part of Ethiopia due to its hardy nature, but it suffers from plant diseases as well as post harvest physiological deterioration (PPD). It has been estimated that cassava farmers, typically resource-poor farmers, lose 48 million tons of fresh root, some 30% of total world production, valued at US$1.4 billion every year to pests, diseases, and PPD (FAO, 2002). The cassava mosaic disease (CMD) pandemic in East and Central Africa, and the cassava brown streak disease (CBSD) in coastal Tanzania, (Legg *et al.,* 1998) severely reduce yields and put rural populations at risk (De Vries and Toeniessen, 2001). Because of its long cropping cycle, 8-24 months, cassava is exposed to an array of pests, diseases and environmental pressures over a prolonged period of time. Therefore, the use of costly inputs, such as pesticides, over the entire crop cycle is prohibitive and uneconomical for the small or large cassava producer.

It has been reported that cassava can produce 250 x 10^3 calories/ha/day compared to 176 x 10^3 for rice, 110 x 10^3 for wheat, 200 x 10^3 for maize, and 114 x 10^3 for sorghum (Cock, 1982). Producing enough food, in a sustainable manner, to meet the needs of an increasing global population is one of the greatest challenges that world face (Anna *et al*., 2010). Recent estimates are that food production will need to double by 2050 (Baulcombe *et al*., 2009). The tropical root crop cassava (*Manihot esculenta* Crantz) is the third most important source of calories for human food in the tropics after rice and maize. Larger proportion of the total cassava production in the world is used as food for humans, with lesser amounts being used for animal feed (Nwokoro *et al.*, 2002) and industrial purposes. An estimated 70 million people obtain more than 500 Kcal per day from cassava and more than 500 million people consume 100 Kcal per day (Kawano, 2003).

Furthermore, the fact that cassava is most often (traditionally) grown on marginal soils robs the plant of important growth enhancing nutrients and exposes the crop to additional stresses making it more susceptible to pests attack leading to more severe crop losses (Catalayud *et al.,* 2002). Among cassava diseases, cassava root rot (*Nattrassia mangiferae*) and stem rots (*Spherostibe repens)* are the most important in different parts of West Africa (Wydra and Msikita, 1998; Hillocks and Wydra, 2002). One of the major constraints of cassava in-ground storage is root rot diseases. Root rot apart from reducing cassava yield can also reduce the quality of cassava root

harvest (Ray and Ravi, 2005). Cassava yield losses up to 80% due to rot diseases have been reported (Theberge, 1985). In some areas, total crop losses have been attributed to rot diseases (Hillocks and Waller, 1997).

Major diseases and insect pests of cassava are caused by cassava mosaic disease (Begomoviruses) (Thresh *et al*., 1994), cassava brown strike virus (CBSV), cassava bacterial blight (*Xanthomonas axonopodis* pv. *Manihotis)* (Vauterin *et al*., 1995), cassava anthracnose disease (*Colletotrichum gloeosporioides* f. sp. *manihotis* Henn. (Penz) Sacc). It is characterized by development of cankers on stems, branches and fruits, and leaf (CIAT, 1972; Chadrasekharan- Nair *et al*., 1979; Makambila and Koumouno, 1994), Cercospora leaf diseases are essentially confined to the foliage where they cause spots and blight: brown leaf spot (BLS) caused by *Mycosphaerella henningsii* (Lozano and Booth, 1974) and insect pests such as cassava green mite (*Mononychellis tanajoa),* cassava mealy bug (CMB) *(Phenococcus manihoti)* and a diversity of weed species. Cassava mosaic disease (Begomoviruses) is the main biotic factor throughout Africa that diminishes production by an estimated 15% to 24% per a year.

A number of fungal diseases of cassava have been reported in some countries of Africa such as republic of Congo, Tanzania, Togo, Nigeria, Uganda and other world parts as follows: *Phytophthora drechsleri* Tucker (Booth, 1978; Theberge, 1985); *Sclerotium rolfsii* (IITA, 1990); *Rosellinia necatrix* Prill (Lozano and Booth, 1976; Booth, 1978); *Fusarium oxysporum* Schlecht, *Botryodiplodia theobromae* Pat, *Aspergillus niger* Van Tieghem, *Aspergillus flavus* Link, *Rhizopus spp; Fusarium solani* (Mart) Sacc., and *Macrophomina phaseolina* (Tassi) Goidanich (Booth, 1978).

Most developing countries economy is based on agriculture including Ethiopia. Cassava (*Manihot esculenta*) is economically and nutritionally useful plant, in south western part of Ethiopia. Plant pathogenic diseases and some insects cause damage and attack on this tuber crop respectively and there by eventually it ends up with a great loss in yield. Cassava (*Manihot esculenta*) is attacked by different plant pathogenic diseases, particularly, by fungi and bacteria in the field conditions. Many research findings on fungal diseases of cassava in different African countries have been reported but nobody has identified so far diseases of cassava in our country. So this study is initiated to isolate, identify and characterize fungal diseases of cassava and to study methods of control in order to increase the yield and thereby support food supply and security in the country. Therefore, it is important to study the diseases that reduce the yield and its productivity as an increase in cassava's yield has significant effect on industrial inputs as a raw material, which subsequently contributes and help in sustainable cassava development in the country. Thus, the general objective of this research is to morphologically characterize fungal *wilt caused by Rhizoctonia spp and Cephalosporium spp.* diseases of cassava (*Manihot esculenta (L.)*) isolates collected from south west part of Ethiopia.

Materials and methods

Sample collection

Diseased cassava samples were collected from different growing sites to isolate and evaluate environmental influences on the pathogens development and the plant growth. Different parts of the cassava were taken; root, stem, leafs, and seed to observe and diagnose symptoms of the disease. Diseased organs of cassava were sampled from five different locations of cassava producing areas: Hawassa/sidama Humbo/Wolayita, W/abaya/Gamogofa, Areka/Wolayita and Jimma zone. Different parts/organs of cassava were collected from the field of cassava farm and subsequently the samples were kept in the refrigerator to isolate the fungal pathogens of cassava.

Isolation of fungal diseases of cassava

During the isolation of fungi from diseased organs, the plant tissues were first washed in sterile distilled water and undergo surface sterilization in 2% NaOCl (Sodium hypochloride) for one minute and then after in 70% alcohol each for one minute. This was followed by rinsing the plant material in sterile distilled water and allowed to dry on sterile tissue paper (Dhingra and Sinclair, 1993; Aneja, 2005; Gesier *et al*., 2005). Next to that, the dried tissues were cultivated on to PDA and incubated at 25^0C to promote growth of mycelium, fruiting bodies or to sporulate out of infected tissue (Roux *et al*., 2004; Gesier *et al*., 2005). Streptomycinsulphate antibiotic was used to avoid the bacterial contamination. WA media was used for sporulation and to have monoconidal isolates of fungi (Gesier *et al*., 2005; Summerell *et al*., 2006). Monoconidial isolates of the recovered fungi were on PDA slants in the test tubes as stock cultures or cultures of fungi and kept in the refrigerator at 4^0C for further studies.

Morphological characterization

Morphological studies were carried out on water agar (WA) medium. Cuts of culture of medium were placed onto PDA medium for 2-3 days. Then, section of cultures was transferred onto WA medium for 7 days in incubator at 25^0C and 12hrs photoperiod. Afterward, morphological observations were taken based on colony, conidia and conidiophores morphology and other morphological characters (Summerell *et al*., 2006).

Data analysis

The statistical analysis of growth characteristics of the isolates at different media, temperature and pH, and mean comparisons of the isolates based on different parameters were conducted using one way ANOVA procedures of SPSS statistical analysis software (SPSS

institute Inc., Cary, NC) version 13. Differences between treatments were determined by using Duncan Multiple Range Test (DMRT) with ($P<0.05$).

Results

As the experimental findings of this research depicts, on the basis of morphological characters, pathogenicity and comparison with the authentic description Kumar *et al*. (2015) the fungus was identified as *Rhizoctonia* spp Variability amongst the isolates was recorded with respect to cultural and morphological characters. From the nine isolates of *Rhizoctonia* spp and eleven isolates of *Cephalosporium* spp isolated, only eight of them were found to have similar characteristics.

Pigment-based Characterization of Cephalosporium spp:

Conidia, produced at tip and collected, road in shape; conidiophores swollen, simple, produced successively. Mycelium, septated, branched with long common hyphae, form net like structures.Culturally, it has aerial hyphae; colony color, purple(edge), black(center), and white(middle) in front side of the plate and gray being brown at center in back side of the plate.

Fig 1. Cultural appreance of *Cephalosporium* spp. (Back (right) and Front (left) side) on MEA after 6 days of incubation at 25^0C.

Isolates of *Rhizoctonia* spp differed with respect to their cultural characteristics. The characteristics viz., type and color of colony, growth rate of fungus and pigmentation were recorded. The nine isolates of *Rhizoctonia* spp had been grown on PDA showed variation in their colony characteristics. Colony color varied from light to dark grey with whitish or brownish tinge. Mostly the colonies had cottony or fluffy mycelial growth with regular to irregular margin (Table 1).

Table 1. Variability in cultural characteristics of different isolates of *Rhizoctonia* spp on Potato Dextrose Agar medium

Isolate	Source/ Location	Colony Characteristics			Mycelial growth after 7 days (mm)
		Type and Color	Shape (Center)	Margin Pattern	
AAUR1	Humbo	Cottony, White	Fluffy	Regular, White	44.60
AAUR2	Areka	White	Fluffy, Greyish	Regular, V-Shaped	43.52
AAUR3	Sidama	White	Cottony	Irregular	42.80
AAUR4	Humbo	Grey	Whitish , Raised	V-Shaped, Irregular	59.5
AAUR5	M. Abaya	White	Suppressed	V-Shaped, regular	35.5
AAUR6	Humbo	White	Fluffy, Greyish	irregular	45.0
AAUR7	Areka	White, Fluffy	Whitish Raised	Suppressed	32.0
AAUR8	Hawassa	White	Cottony	Regular	48.0
AAUR9	Jimma	smoky grey	White, suppressed	Regular, V-Shaped	43.5

**Mean of 9 observations, AAUR:= Addis Ababa University Rhizoctonia spp*

Isolate AAUR-1, AAUR-3, AAU R3, AAU R5 , AAU R6, AAU,R7 and AAU R9 had whitish appearance. The fluffy growth was observed in AAUR7 isolates. AAUR-6 and AAUR-7 had fluffy at the center too. Cottony growth was seen in AAUR-1. Among the studied colonies, suppressed growth was observed in AAUR5 and AAUR-9, while fluffy central pattern was depicted on isolate AAUR-1 AAUR2 and AAUR-6.

From all the colonies studied, raised type of colonies was observed in AAUR-4, and AAUR-7isolates. Differences had been exhibited among the isolates in terms of colony colors (Table 1). White colonies were observed in all isolates except three isolates, viz., AAUR-4, and AAUR-9 which exhibited at least slight greyish color,. Isolate AAUR-4 and AAUR-9 had shown light brown with white greyish centre.On the other hand, the colony margins varied from regular to irregular (Table 1). Regular margins were observed in five isolates viz., AAUR-1, AAUR-2, AAUR-5, AAUR-8, AAUR-8, and AAUR-9 whereas, three isolates, namely, AAUR-3, AAUR-4, and AAUR-6 had irregular margins. Margins were whitish in AAUR-1 isolates. Besides, V-shaped margins were observed in isolate AAUR-2, AAUR-4 and AAUR-5 and AAUR-9. In isolates AAUR-1, AAUR-3, and AAUR-4, the margins were had dull white color. The mycelia growth of isolates 7 days after inoculation ranged from 32.0 to 48.0 , in AAU-R 7 and AAU-R 8, respectively.

Pigment-based Characterization of Rhizocotonia spp

Asexua fruit bodies and spores lacking. Mycelium, septated, repeatedly branched, thick, form buds and hyphae are interlocked. Culturally, lacks aerial hyphae; colony color, purple, smooth in front side of the plate and yellow in back side of the plate (Fig 2).

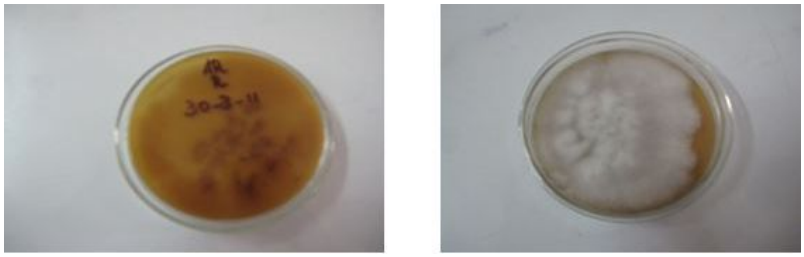

Fig 2. Cultural appearance of *Cephalosporium* spp (Back (right) and Front (left) side) on MEA after 6 days of incubation at 25^{0}C

The eleven isolates of *Cephalosporium* spp were grown on PDA and showed variation in their colony characteristics. Colony color varied from light to dark grey with whitish or brownish tinge. Mostly the colonies had cottony or fluffy mycelial growth with regular to irregular margin (Table 2).

Table 2. Variability in cultural characteristics of different isolates of *Cephalosporium* spp on Potato Dextrose Agar medium

Isolate	Source/ Location	Colony Characteristics			Mycelial growth after 7 days (mm)
		Type and Color	Shape (Center)	Margin Pattern	
AAUCPH9	Humbo	Dull White	White	Suppressed, White	40.2
AAUCPH10	Humbo	White, Grey	Cottony	Regular	43.5
AAUCPH11	Humbo	White, Fluffy	Brown	Irregular	49.0
AAUCPH12	Areka	Grey	Suppressed	Regular	42.5
AAUCPHl3	Areka	Cottony White,	Greyish	Irregular	45.5
AAUCPH14	Areka	Dull White	Light Grey, Fluffy	Regular	44.0
AAUCPHl5	Areka	White	Fluffy	Regular	42.5
AAUCPH16	Jima	White, Fluffy	Greyish	Regular, White	45.0
AAUCPH17	Jima	Light Brown	Greyish	Irregular	40.0
AAUCPH18	M. Abaya	Light Grey	White Tinge, Raised	Regular	43.5
AAUCPHl9	Hawassa	white	Grey , Fluffy	Irregular	45.5

**Mean of 11 observations, AAUCp:= Addis Ababa University Cephalosporium spp*

The fluffy growth was observed in two isolates viz., AAU-cph-11, and AAU-cph-16. Conversely, AAU-Cph-9, AAU-cph-14 and AAU-cph-19 had white appearance whereas grayish growth was observed in three isolates viz., AAU-cph-10, AAU-cph-12, and AAU-cph-18. Among the studied colonies, suppressed growth was observed in AAU-cph-12 and v-shape pattern was depicted on none of the isolates. From all the colonies studied, grey centered type of colonies was observed in AAU-cph-13, AAU-cph-14, AAU-cph 16 and AAU-cph-17 isolates. Raised center was observed in AAU-cph18.

Variation had been exhibited among isolates in terms of colony colors (Table 1). White colonies were observed in all isolates except three isolates, viz., AAU-cph-18, AAU-cph-17 and AAU-cph-12 which exhibited at least slight greyish color. Isolate AAU-cph-17 had shown light brown with white greyish centre. The colony margins varied from regular to irregular (Table 1). Regular margins were observed in eleven isolates viz., AAU-cph-10, AAU-cph-12, AAU-cph-14, AAU-cph-15, AAU-cph-16, and AAU-cph-18. Whereas, eight isolates, AAU-cph-11, AAU-cph-13, AAU-cph-17 and AAU-cph-19 had irregular margins. Margins were whitish in AAU-cph-1, AAU-cph-9, and AAU-cph-16 isolates. Besides, white margins were observed in isolate AAU-cph-9, and AAU-cph-16, in which the margins were had dull white color. The mycelia growth of isolates 7 days after inoculation ranged from 40.2 to 49.0 , in AAU-cph 9 and AAU-cph 11, respectively.

Cultural Appreance

Cultural appreance of the identified genera after 7 days of incubation at 25 ^{0}C. *Botyrits* spp. (from back side of the plate) & from front side of the plate *Hendersanula* spp (Front side back side). Cultural appreance of the identified genera after 7days of incubation at 25 ^{0}C. *Botyrits* spp. (from back side of the plate) & from front side of the plate *Hendersanula* spp (Front side back side). *Acrostalagmus* spp. (back side of the plate) & *Pullularia* spp. (back side) (Figure 3).

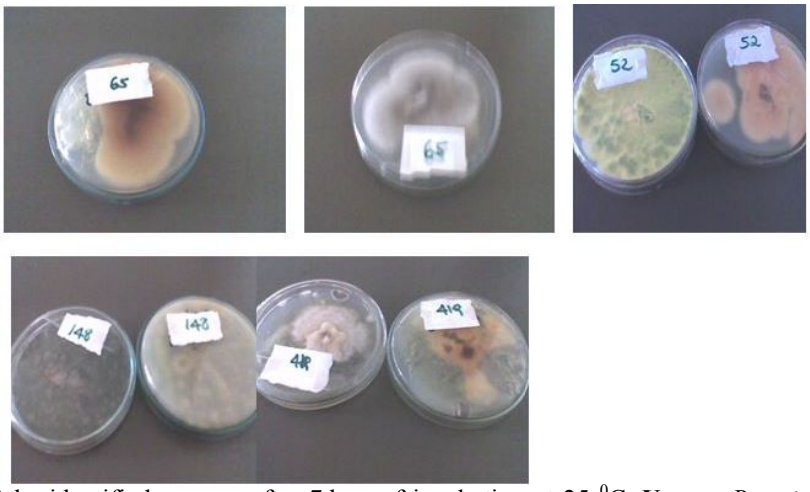

Fig 3. Cultural appreance of the identified genera after 7days of incubation at 25 ^{0}C. **Upper**: *Botyrits* spp. (from back side of the plate) & from front side of the plate *Hendersanula* spp (Front side back side). **Bottom**: *Acrostalagmus* spp. (back side of the plate) & *Pullularia* spp. (back side)

Table 3. Summary on cultural characteristics of *Rhizoctonia* and *Cephalosporium* spp. on MEA after 7days of incubation at 25^{0}C

Isolate	Colony Color and Condial Structure	Substrate color	Colony dia meter (mm)	Sporul ation	Aerial hyhpae
***Rhizoctonia* spp.**	purple,smooth in front side of the plate and yellow in back side of the plate. Asexua fruit bodies and spores lacking.	Light brown	81.6	+	a
***Cephalosporium* Spp.**	Conidia, produced at tip and collected, road in shape; conidiophores swollen, simple, produced successively aerial hyphae; colony color, purple(edge), black(center), and white(middle) in front side of the plate and gray being brown at center in back side of the plate.	Brown	81.3	+++	p

(Key: - + Poor sporulation: 1-10 spores; a: aerial hyphae absent; p: presence of aerial hyphae; +++ Good sporulation: More than 100 spores).

In *Rhizoctonia* spp., the asexual fruit bodies and spores are lacking. Mycelium was septated, repeatedly branched, thick, form buds and hyphae were interlocked. Culturally, most of the isolates lack aerial hyphae; colony color, purple, smooth in front side of the plate and yellow in back side of the plate.

In *Cephalosporium* Spp., the conidia, produced at tip and collected, was rod in shape; conidiophores swollen, simple, produced successively. Mycelium was septated, branched with long common hyphae, form net like structures.Culturally, it has aerial hyphae; colony color, purple(edge), black(center), and white(middle) in front side of the plate and gray being brown at center in back side of the plate.

Discussion

In the this experiment, the isolates of cassava wilt disease were characterized and by using common mold identification manual based on their morphological features. The fungal pathogens of cassava, *Rhizoctonia* spp., *Cephalosporium* spp., *Pullularia* spp., *Hendersonula* spp., *Botrytis* spp. and *Acrostalagmus* spp. Burnet and Hunter (1972 and 1998) have reported that these pathogens have a capacity to infect and cause a disease to plants. Studies so far conducted indicated that a number of fungal diseases of cassava have been reported in some countries of Africa such as republic of Congo, Tanzania, Togo, Nigeria, Uganda and world parts, *Phytophthora drechsleri* Tucker (Booth, 1978; Theberge, 1985); *Sclerotium rolfsii* (IITA, 1990); *Rosellinia necatrix* Prill (Lozano and Booth, 1976; Booth, 1978); *Fusarium oxysporum* Schlecht, *Botryodiplodia theobromae* Pat, *Aspergillus niger* Van Tieghem, *Aspergillus flavus* Link, *Rhizopus spp; Fusarium solani* (Mart) Sacc., and *Macrophomina phaseolina* (Tassi) Goidanich (Booth, 1978). For this study, only the two pathogens of cassava, *Rhizoctonia* spp. and *Cephalosporium* spp. were selected based on their pathogenicity, infection

and disease development on the host. *Rhizoctonia* spp. was isolated from Areka/Wolayita, West Abaya/Gamogofa and Humbo/Wolayita, and *Cephalosporium* spp. was isolated from Humbo/Wolayita and West Abaya/Gamogofa zones. Their infection severity was high on cassava seedlings, leaf and stem cuttings that were incubated at 25^0 C in laboratory under controlled conditions. *Rhizoctonia* spp. has a capacity to infect and devastating plants (Hawks worth *et al*., 1995). It has been observed that cassava yield losses up to 80% due to rot diseases have been reported (Theberge, 2006). Similarly, it has indicated, in some areas, total crop losses have been attributed to rot diseases (Hillocks and Waller, 1997). Lozano and Booth, (1974) have reported that *Cercospora* leaf diseases are essentially confined to the foliage where they cause spots and blight: brown leaf spot (BLS) caused by *Mycosphaerella henningsii*.

Morphologically, *Rhizoctonia* spp. was septated, repeatedly branched, thick, form buds and hyphae are interlocked. Asexua fruit bodies and spores lacking. This collabotarated with the reported that *Rhizoctonia* De Candolle anamorph (asexual stage) classification belongs to the Mitosporic fungi (Syn.: Deuteromycotina, Deuteromycetes, fungi imperfecti, asexual fungi) (Hawks worth *et al*., 1995).

Conclusion

In conclusion, characterization of six fungal genera of cassava diseases collected from the southwest of Ethiopia were reported to have a varied pigmentation, marginal characteristics and mycelia growth. The distribution, infection, severity and frequency of *Rhizoctonia* spp. and *Cephalosporium* spp. are more than the remaining diseases of cassava. In general, *Cephalosporium* spp. was more diverse than *Rhizoctonia* spp. in morphological characteristics. towards change in environmental factors and cultural media. Each genus had different time of onset of diseases symptom development. Except *Acrostalagmus* spp. and *Hendersonula* spp., all genera were causative agents of systematic diseases. From the result that *T. viride* exhibited higher percent of inhibition (46.4%) than *T. harzianum* (22.6%), it is possible to conclude that *T. viride* is more effective than *T. harzianum* in controlling cassava diseases caused by *Rhizoctonia* spp. and *Cephalosporium* spp.

Recommendations

From the findings of the research, it the government is recommended to initiate a large scale assessment of cassava and disease characterization for it is a very good source of food security in several regions of the country. Universities and research institutions shall embark on additional researches that focus on production, post harvest technology, processing, marketing and determination of quality of cassava across the southern regions of the country. More researches should be done on ecology and diversity of *Rhizoctonia* spp and other fungal diseases of cassava in the country. In this work, some fungal genera have showed organ specificity in the development of diseases symptoms; which need further studies to answer the question why. Further intensive characterization and diseases controlling methods of these fungal genera requires more researches to be done on a larger scale.

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