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Reducing Potato Cyst Nematode Attack on Granola L. with in-Vitro and ex-Vitro G0 Seed Production

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Abstract.

Potato Cyst Nematode/PCN *(Globodera rostochiensis)* attack *is the most critical risk in potato farming.* The attack was marked by a decrease in the production of up to 60%. The objectives of this study were to reduce postharvest losses due to PCN attacks on Granola L and to compare yields from G2 and G0 seeds. This research was conducted in 5 districts (N1, N2, N3, N4, and N5) in Wonosobo Regency, Central Java, Indonesia. The method used in this study was the comparison of yields from G2 and G0 seeds in the same area, considering temperature (V1) and altitude (V2). The results showed that Granola L from G2 seeds for 100 days after planting was 15.9 tons/ha, while the results for G0 seeds in the same field increased to 12.18 tons/ha. In G2 seeds, 52 cysts/100 ml of soil were obtained, while in G0 seeds, cysts were not accepted. Combining G0 seeds in vitro and ex-vitro on Granola L can reduce PCN attacks and increase yields by up to 73%. Conducting experiments with other cultivars, such as the Atlantic and Agria, is necessary for further research.

Keywords; Ex-vitro, Granola L, In-vitro, Potato cyst nematode.

Minimasi Serangan Nematoda Sista Kentang pada Granola L. dengan Produksi Benih In-Vitro dan Ex-Vitro G0

Abstrak

Serangan Nematoda Sista Kentang/NSK (Globodera rostochiensis) merupakan risiko paling kritis pada budidaya kentang. Serangan tersebut ditandai dengan penurunan produksi hingga 60%. Tujuan penelitian ini adalah untuk mengurangi kerugian pasca panen akibat serangan NSK pada Granola L dan membandingkan hasil benih G2 dan G0. Penelitian ini dilakukan di 5 kabupaten (N1, N2, N3, N4, danN5) di Kabupaten Wonosobo, Jawa Tengah, Indonesia. Metode yang digunakan dalam penelitian ini adalah perbandingan hasil benih G2 dan G0 pada lahan yang sama dengan mempertimbangkan suhu (V1) dan ketinggian tempat (V2). Hasil penelitian menunjukkan Granola L dari benih G2 selama 100 hari setelah tanam sebanyak 15,9 ton/ha, sedangkan hasil benih G0 pada lahan yang sama meningkat menjadi 12,18 ton/ha. Pada benih G2 diperoleh kista sebanyak 52 buah/100 ml tanah, sedangkan pada benih G0 kista tidak diterima. Penggabungan benih G0 secara in vitro dan ex-vitro pada Granola L dapat mengurangi serangan PCN dan meningkatkan hasil hingga 73%. Melakukan percobaan dengan kultivar lain, seperti Atlantik dan Agria untuk penelitian lebih lanjut.

Kata kunci; Ex-vitro, Granola L, In-vitro, Nematoda Sista Kentang**.**

Introduction

Potatoes *(Solanum tuberosum* L.*)* are the most critical food horticulture in the world after wheat, rice, and corn. Potatoes are produced in around 310 million tons annually (Hardjomidjojo *et al*., 2022). Indonesia is the world's 14th producer and largest producer in Southeast Asia, with an average growth rate of 8.4% per year with an average production of 1.09 million tons/year (Yusianto *et al*., 2023). Indonesia's most significant average production in 2018-2022 in Central Java was 277,702 tons; West Java was 277,030 tons; East Java was 260,714 tons (Figure 1).

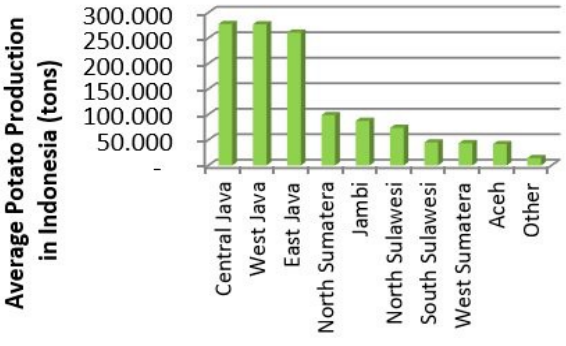

Figure 1. Average Potato Production in Indonesia

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If further specified, Central Java has two dominant potato-producing regions, namely Banjarnegara, 124,484 tons, and Wonosobo, 54,265 tons, both in the Dieng plateau. The most popular potato variety on the Dieng plateau is Granola L. This type of potato dominates 90% of Indonesian potato farming (Wibowo *et al*., 2019).

The most critical risk in potato farming in the field, storage, and transportation logistics is the Potato Cyst Nematode [(*G. rostochiensis*) (Delate & Dewitt 2004; Griffin *et al.* 2015; Nagachandrabose 2020)]. They are the most destructive pathogens for potato plants (Aires *et al*., 2009). The attack was marked by a decline in production of up to 60%. When the population in an area is very high, the yield decrease can reach 80%. These nematodes were found to start destroying potato plants in Central Java, Indonesia, around 2003 (Supramana *et al*., 2019).

This pathogen was first discovered in Indonesia in March 2003 in Tulungrejo, Malang, East Java (Indarti *et al*., 2004). This pathogen was found in West Java and Central Java, including the Dieng plateau. Lisnawita (2017) has identified molecularly the nematode species in Central Java as *G. rostochiensis*. The stages of this nematode cycle start from the egg, larval, and adult stages and require 38-48 days. Female nematodes are amphimictic reproduction, globose, sessile, and motile (Muthulakshmi *et al*., 2012). The male is shaped like a worm. The life cycle is 5-7 weeks, depending on environmental conditions, producing 200-500 eggs. Nematodes will form cysts in environmental conditions with no hosts and very low and very high temperatures (Viaene 2015; Janssen *et al.* 1995). Nematodes will be active after ecological conditions are suitable and there are host plant roots (Mulyadi *et al.* 2003; Mugniéry 2008; Dutt *et al.* 2014). This cyst can last more than 30 years. Adult male nematodes are not plant parasites, but their role in the propagation of nematodes is enormous because they are very active in marrying females (Sereno & Danchin, 2014). If there is enough nutrition, many of the larvae become females, but if the supply of nutrients decreases often, there is a process of sex reversal; the larvae that will become females turn into males.

PCN infection in the vegetative phase with a high population level results in stunted plant growth, yellowing, wilting, and necrosis. Small tubers can be produced in the generative phase, and cysts will appear attached if the plant is removed. Some features of this nematode attack are that the lower leaves of the potato begin to turn bright yellow at the age of 40-50, spread to the upper leaves, and die around 70 days after planting. A small yellow cyst is attached along the roots if the yellowing plants are removed (Figure 2).

Figure 2. The Potato Cyst Nematode *(G. rostochiensis)*

Currently, farmers in Central Java, Indonesia, use traditional methods using G2 seeds (commercial), where G2 is a G0 (primary) derivative planted outside the greenhouse. Whereas G0 seeds are produced from in vitro culture as plantlets, the knot as a multiplication organ is then acclimatized and propagated ex-vitro. Using G2 seedlings in the PGN attack area has a high risk of pest attack and causes high postharvest losses. Therefore, we need good potato seeds that are free of viruses. One of them is G0 seeds produced from in-vitro culture in the form of plantlets, where through in vitro techniques, using the knot as multiplication organ acclimatized and propagated ex-vitro.

This paper showed how to reduce yield losses from PCN attacks on Granola L varieties, focusing on seed production and yield. We use a combination of in vitro and ex-vitro. In vitro seed multiplication in bottles was carried out in a culture laboratory while ex vitro with cuttings technique was performed in a screen house. As a testing environment, we selected farmer fields in the Dieng Plateau in Banjarnegara District and

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Wonosobo, Central Java, one of Indonesia's best potato producers.

Method

In producing G0 seeds by in vitro culture techniques, explant growth must be conditioned in an aseptic and controlled environment to optimize the presentation of potato plantlets. The research material consists of (1) an incubation room of 30 m2; (2) a culture rack; (3) an autoclave; (4) TL
40-Watt lamp; (5) glassware; (6) a culture bottle; and (7) sterile knife. G2 seeds come from the selection of G0 seeds (primary). G2 seeds, indeed, do not contain PCN.

Parent plants need to be considered in the in vitro method. In this research, the Granola L. variety was used. It was ascertained to be pathogen-free, especially the PCN virus. Virus-free testing was carried out on plantlets using serological tests. After isolation and the system has grown into plantlets, it is propagated or propagated by cutting the nodal with a sterile knife. Growth of cuttings in vitro until subcultures can be carried out between 3-5 weeks after planting or inoculation (Ibrahim *et al*., 2016). Then, acclimatization will be carried out in the Screen house. Stages in vitro and acclamation of G0 seeds can be seen in Figure 3. We planted plantlets in a greenhouse on potato plants; one plantlet was grown in soil media with 5-10 plants. The media used are manure and soil that has been sterilized with cocopeat and charcoal husk in a ratio of 2:1.

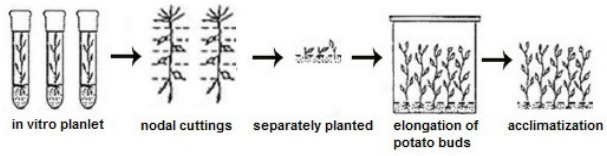

Figure 3. Stages of the in-vitro and ex-vitro acclamation

This study used Granola L varieties; one culture bottle contained ten plantlets. The study results in the percentage of life acclimatized to 99% by maintaining healthy conditions and free of fungal/bacterial/viral contamination.

Figure 4. The culture bottle consists of 10 plantlets

Seeds produced by the in Vitro culture method are maintained on plantlets and never exposed to soil. So, the seed potatoes grown are also healthy, meaning that the seeds do not contain diseases such as viruses, bacteria, or fungi. Healthy potato seeds are very supportive of increasing potato production. The tissue culture method is very supportive for quickly producing healthy seeds in large quantities. After being acclimated, it is planted ex vitro in the screen house, as seen in Figure 5.

Figure 5. Seeds that are ready to plant screen houses

G2 seedlings were planted in an area of 1 ha in the Kejajar (N1), Garung (N2), Mojotengah (N3), Kalikajar (N4), and Sapuran (N5) sub-district in Wonosobo district, Central Java, Indonesia can be seen in Figure 6.

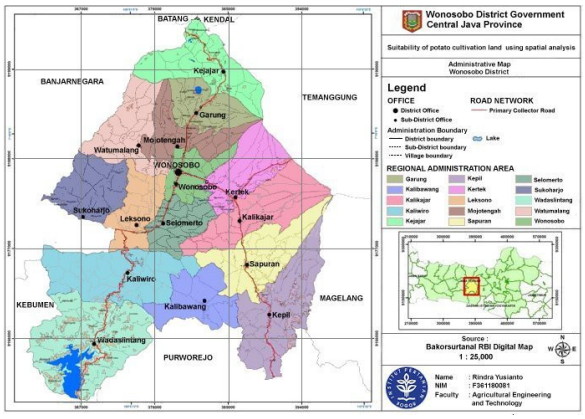

Figure 6. Wonosobo Administration Map

One hundred days after planting were observed to obtain an average yield, and 100 ml soil samples were celebrated after harvest. Then, the land is cleared and sterilized to

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ensure no PCN in the soil content. Soil samples were examined in the Adhiguna laboratory in Kejajar, Wonosobo, Central Java, Indonesia. After ascertaining that there is no PCN, the land is processed to be ready for planting with G0 seedlings. The same treatment is observed 100 days after planting to obtain an average yield and celebrated
100ml soil samples after harvest.

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RESULT

PCN attacks in the Dieng plateau are almost evenly distributed. PCN is found at an altitude of 1577 to 2047 m above sea level with a soil temperature between 15-24 °C. The research results show significant differences. Experimental gardens in Kejajar (N3) have relatively lower temperatures and higher heights. The results showed that Granola L from G2 seeds for 100 days after planting was 15.6 tons/ha.

Based on the spatial analysis, only the N1 sample has an altitude above 1,300 m ASL. Altitude maps based on spatial analysis are shown in Figure 7.

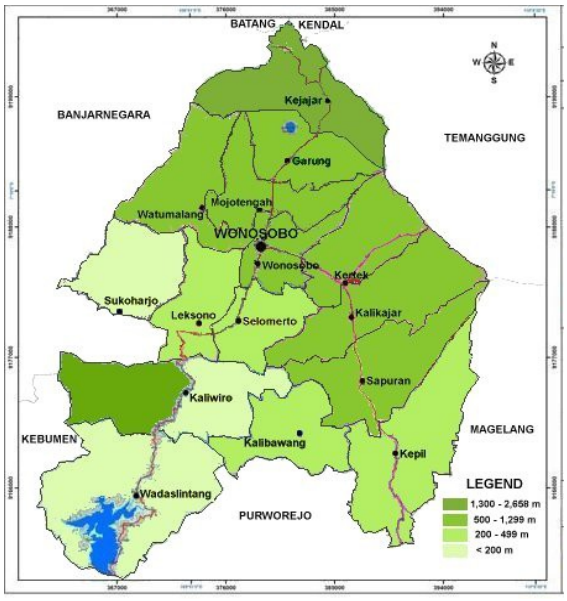

Figure 7. Altitude map based on spatial analysis

The average temperature of 20 ^{0}C-30 ^{0}C is in 5 samples—only sample N1 with a temperature of 17.7 ^{0}C. Temperature maps based on spatial analysis are shown in Figure 8.

Figure 8. Temperature map based on spatial analysis

We determined the cropland suitability level for five samples, taking into account the altitude (V1) and Temperature (V2). We used intervals of 3.75 and 4 classes to analyze cropland suitability.

Table 1. Suitability level interval for potato cropland

Class	Crop Land suitability level	Total Value	Description
S_1	The most suitable	> 13.3	Excellent condition
S_2	Suitable	10.2 – 13.2	Good condition
S_3	Rather suitable	7.1 – 10.1	Poor condition
NS	Unsuitable	4.0 – 7.0	Deplorable condition

Based on cropland suitability level interval, cropland suitability classes can be as follows (Table 2):

Table 2. Cropland suitability classification

Sample Code	Location	Result	
		Total Value	Suitability level
N_1	Tieng, Kejajar	17	Very Suitable
N_2	Jengkol, Garung	16	Suitable
N_3	Deroduwur, Mojotengah	15	Suitable
N_4	Tegalombo, Kalikajar	15	Suitable
N_5	Ngadisalam, Sapuran	16	Suitable

Five sub-districts in Wonosobo produce potatoes, as in Table 3.

Table 3. Current potato production in the Wonosobo district

Sample Code	Sub-district	Current Potatoes production (ton)
N_1	K	447,060
N_2	G	74,240
N_3	Mojotengah	905
N_4	Kal	19,683
N_5	Sa	661

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From Table 1, it can be seen that sample N1 has the highest production, namely 447,060 tons/year, while sample N5 has the lowest display, 661 tons/year. N1 is in the northern part of the study area, while N5 is in the southern region.

A comparison of yields using G2 seeds in the experimental land in Kuripan and Kejajar can be seen in Table 4.

Table 4. Total harvest with G2 seeds

Sample Code	Location	Altitude (m asl)	Temperature (°C)	Yield (tons/ha)
N_1	Tieng, Kejajar	1,378	19	17.7
N_2	Jengkol, Garung	1,019	23	15.5
N_3	Deroduwur, Mojotengah	860	24	15.7
N_4	Tegalombo, Kalikajar	815	23	15.8
N_5	Ngadisalam, Sapuran	760	26	14.8

We compared the suitability level with the presence of PCN in each sample. In G2 seeds, 52 cysts/100ml of soil were obtained, while in G0 seeds, cysts were not accepted. The distribution of PCN can be seen in Table 5.

Table 5. Distribution of PCN and the number of cysts per 100 ml of soil

Seed	Soil observed	Duration of Observation	The number of cysts
G2	100 ml	100 daps	52
G0	100 ml	100 daps	0

We observed the Granola L variety under the same conditions 100 days after planting with G2 and G0 seeds. After two planting periods, it can be seen that by using G0 seeds, the soil under observation does not contain cysts.

We collected data with a duration of 3 times the harvest period. We compared the yields using seed G0 and seed G2. Then, we measured the presence of PCN for each sample plot. A comparison of products using G0 seeds can be seen in Table 6.

Table 6. Total harvest comparison

Sample Code	Location	G0	G2
		Yield (tons/ha)	Yield (tons/ha)
N_1	Tieng, Kejajar	19.8	17.7
N_2	Jengkol, Garung	16.5	15.5
N_3	Deroduwur, Mojotengah	17.6	15.7
N_4	Tegalombo, Kalikajar	19.7	15.8
N_5	Ngadisalam, Sapuran	16.8	14.8

The results showed that Granola L. from G0 seeds in the same field increased to 12.18 tons/ha. Table 4 shows that the most significant harvest increase was in the N4 sample, namely an increase of 3.9 tons/ha after using G0 seeds. Meanwhile, N2 experienced the smallest growth in production, namely 1 ton/ha.

It shows that the variable altitude and temperature have an effect on PCN attack and have a significant impact on increasing production when using different seeds.

Discussion

The results showed that at the location of samples with soil temperatures between 15-16 °C, the number of cysts was higher, namely 50-52, while at sample locations with higher soil temperatures of 20–24 °C, there were no cysts. Soil temperature affects the development of PCN. The optimum soil temperature is 15–21°C. In the use of G2 seedlings, 52 PCN cysts were found from 100 ml of soil after harvest, while G0 seedlings were not found. The future spread of PCN in Kejajar (N1) has great potential because the area has the highest altitude compared to other sites where nematodes have not attacked. This active nematode movement is only a few cm/year, but with the help of natural conditions, this nematode can spread in a short time.

Chlorosis leaves appeared on the potato planting field 70-80 days after planting, which was attacked by nematodes with relatively high populations. The yellowing of the potato garden is only widespread in some of the study areas. When the yellowing agent is removed, the yellow female PCN nematode appears attached to the potato root.

Some researchers previously stated that crop rotation is the way to control this nematode. Combining the processes of susceptible potato varieties, resistant varieties, varieties with early yield characteristics, and non-host plants managed this nematode. Rotating plants with twice- planting resistant varieties followed by non- host plants and then susceptible plants combined with nematicides is also effective in controlling this nematode. However, good seeds derived from G0 tissue culture with in vitro and ex vitro techniques can reduce the risk of this type of nematode attack. By using nematode-free seedlings, postharvest yields are further increased.

Research Limitation

Granola L is the most dominant potato cultivar in Wonosobo, Central Java. Granola L is used not only for vegetable potatoes but also for the potato chips and potato stick industry. Based on Pavlista (2011), better cultivars for this industry are Atlantic and Agria. Atlantic is not well-cultivated in the Dieng Plateau because this variety is very susceptible to pests, including nematodes, while Agria has started to be cultivated mainly for industrial potatoes. This study's limitations are that it does not discuss Atlantic and Agria potato varieties related to PCN attacks and yields based on G2 and G0 seeds.

Conclusion and Recommendation

In this study, we compared the performance of Granola L cultivars derived from G0 and G2 seedlings in field trials at representative locations in the potato planting area in Kejajar and Kuripan in the Dieng Plateau, Central Java. In this experiment, Granola L cultivars derived from G0 seedlings in vitro and ex-vitro showed better potential results when compared to similar cultivars from G2 seedlings. In addition, the number of PCN cysts in the observed soil showed that more PCN cysts were found at lower temperatures with higher altitudes, especially those using G2 seeds. For seeds originating from in vitro and ex-vivo, G0 seeds and PCN cysts were not found after harvesting. The results showed that Granola L from G2 seeds for 100 days after planting was 15.6 tons/ha, while the results for G0 seeds in the same field increased to 27 tons/ha. In G2 seeds, 52 cysts/100ml of soil were obtained, while in G0 seeds, cysts were not accepted. Combining in vitro and ex-vitro G0 seeds on Granola L can reduce PCN attacks and increase yield by up to 73%.

Conducting experiments with other cultivars, such as the Atlantic and Agria, is necessary for further research.

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