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Preface

We would like to present, with great pleasure, the inaugural volume-9, Issue-7, July 2023, of a scholarly journal, *International Journal of Environmental & Agriculture Research*. This journal is part of the AD Publications series *in the field of Environmental & Agriculture Research Development*, and is devoted to the gamut of Environmental & Agriculture issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

This journal was envisioned and founded to represent the growing needs of Environmental & Agriculture as an emerging and increasingly vital field, now widely recognized as an integral part of scientific and technical investigations. Its mission is to become a voice of the Environmental & Agriculture community, addressing researchers and practitioners in below areas.

Environmental Research:

Environmental science and regulation, Ecotoxicology, Environmental health issues, Atmosphere and climate, Terrestric ecosystems, Aquatic ecosystems, Energy and environment, Marine research, Biodiversity, Pharmaceuticals in the environment, Genetically modified organisms, Biotechnology, Risk assessment, Environment society, Agricultural engineering, Animal science, Agronomy, including plant science, theoretical production ecology, horticulture, plant, breeding, plant fertilization, soil science and all field related to Environmental Research.

Agriculture Research:

Agriculture, Biological engineering, including genetic engineering, microbiology, Environmental impacts of agriculture, forestry, Food science, Husbandry, Irrigation and water management, Land use, Waste management and all fields related to Agriculture.

Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with *IJOEAR*. We are certain that this issue will be followed by many others, reporting new developments in the Environment and Agriculture Research Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOEAR* readers and will stimulate further research into the vibrant area of Environmental & Agriculture Research.

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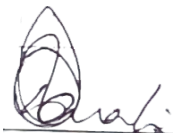

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Occurrence of the Distemper Canine: Ultrastructural and Histophatological Aspects

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***Abstract*—** *Canine distemper caused by the paramyxovirus of the genus Morbilivirus is a highly contagious disease that mainly affects dogs of all ages, with an important socioeconomic impact on the Brazilian veterinary field due to the high cost of treatment, failures in vaccine immunization and the suffering of animals and owners. It presents itself in 2 forms, overacute, characterized by abrupt fever and sudden death and, acute, when the animals show signs of fever, prostration, lack of appetite, nasal and ocular secretions, conjunctivitis, vomiting, respiratory distress, anorexia, diarrhea, dehydration, and cutaneous rash, which may follow neurological symptoms such as paralysis, convulsions and death. From 2004 to 2015, approximately 622 samples from dogs with suspected distemper were sent to the Electron Microscopy Laboratory of the Instituto Biológico, São Paulo, SP, Brazil (oral and nasal swabs, feces, urine and organ fragments). for diagnosis of viral agents. The samples were processed using transmission electron microscopy (negative staining and resin embedding) and routine histopathological techniques. In the transmission electron microscope, 254 samples (40.83%) were visualized, pleomorphic, rounded or elongated, enveloped paramyxovirus particles containing helical herring-bone like nucleocapsid, measuring between 100 and 500 nm in diameter. In ultrathin sections of the brain, the presence of a nucleus with marginalized chromatin containing intranuclear inclusions was visualized. Intracytoplasmic granular amorphous inclusions, formed by viral nucleocapsids were also observed. Complete particles measuring 100 to 250 nm in diameter and incomplete particles, measuring on average 70 nm in diameter, budding from the plasma membrane could also be identified. Large areas of demyelination were also observed. Histological sections of the brain showed perivascular and focal mono and polyclonal encephalitis, monoclonal meningitis, congested blood vessels and parenchyma with areas of demyelination. The presence of eosinophilic inclusion bodies was also observed in several nerve cells (Corpuscles of Lentz).*

***Keywords*— Canine distemper, dogs, Transmission Electron Microscopy, Histopathology.**

I. INTRODUCTION

Canine distemper is a highly contagious disease that mainly affects dogs of all ages. The disease is caused by a paramyxovirus belonging to the *Paramyxoviridae* family of the genus *Morbilivirus*. This genus includes, in addition to canine distemper virus (CDV), rinderspest virus (RPV), peste des petits ruminants virus (PPRV) and measles virus (MV) (Grifin, 2007). Paramyxoviruses (PMVs) are enveloped particles, with nucleocapside helicoidal and negative-sense, single-stranded RNA viruses with genes coding for at least six major proteins, nucleocapsid (N), phosphoprotein (P), matrix (M), fusion glycoprotein (F), receptor binding protein (RBP, formerly designated variously as HN, H, or G), and the large protein (L) that possesses RNA-dependent RNA polymerase (RdRp) activity (Rima et al., 2019). CDV has a tendency to cross the species barrier by infecting species other than *Canidae*, such as *Mustelidea*, *Procyonidae*, *Ursidae* and *Viverridae* (Greene et al., 2012).

Phylogenetic studies suggest that paramyxoviruses related to human mumps virus (MuV, *Rubulavirus*) and canine distemper virus (CDV, *Morbillivirus*) in host bats may have the potential to create spillover events and cause disease in other mammalian species (Drexler et al., 2012).

The disease has a high socioeconomic impact in the Brazilian veterinary field due to the high cost of treatment, failures in vaccine immunization and the suffering of animals and guardians (Headley et al., 2012). In the wild, it is considered an emerging disease, since wild canids are vulnerable (Jucá et al., 2022), such as lions, tigers, leopards (Appel et al., 1994) and monkeys (Sun et al., 2010). The disease has also been described in aquatic mammals and cetaceans such as dolphins (Stone et al., 2011). In the United States, the occurrence of contamination from domestic dogs to raccoons indicates that they can act as intermediate hosts (Kapil et al., 2008). In other countries such as Italy, distemper represents an immediate problem due to the illegal trade in puppies from east Europe (Dall'ara, 2020; Mira et al., 2018).

The disease presents in 2 forms, overacute, characterized by fever and sudden death, and acute, when the animals show signs of fever, prostration, lack of appetite, nasal and ocular secretions, conjunctivitis, vomiting, respiratory distress, anorexia, diarrhea, dehydration, and cutaneous rash, in addition to neurological symptoms such as paralysis, seizures and death (Oliveira et al., 2008).

Histologically, canine distemper virus produces necrosis of lymphatic tissues, interstitial pneumonia, and cytoplasmic and intranuclear inclusion bodies in respiratory, urinary, and gastrointestinal epithelium, neuronal degeneration, encephalitis, meningitis and intranuclear inclusion bodies (Origgi et al., 2012). The duration and severity of the disease depend on factors such as the animal immune status, strain virulence, affected organs, among others. The course can be short (10 days) or go on for weeks or months. The mortality rate ranges from 30 to 80% and surviving animals may recover normally or have permanent central nervous system sequelae or late complications such as demyelinating encephalitis (old dog encephalitis), ascending paresis, paralysis, convulsions and hyperkeratosis of the paws, which can lead to the death of animals. Young dogs are more susceptible, but everyone regardless of age can be affected. Dogs that recover from acute disease with persistent infection may shed virus in urine and through the skin on the foot pads. Transmission occurs through aerosols or contaminated food and objects (Beineke et al., 2009; Martella et al., 2008; Kapil & Yeary, 2011).

This work aimed to investigate the presence of paramyxovirus in samples of feces, urine, ocular and nasal swabs and organ fragment from dogs, using transmission electron microscopy and histopathology techniques.

II. MATERIAL AND METHODS

2.1 Clinical cases

During the period from 2004 to 2015 samples of feces, urine, ocular and nasal swabs and organ fragments from 622 dogs, from the State of São Paulo, SP, Minas Gerais, MG, Espírito Santo, ES and Rio de Janeiro, RJ, Brazil, were sent to the Electron Microscopy laboratory of the Biological Institute of São Paulo, SP, Brazil for research of viral agents. The dogs were of different breeds, of both sexes and aged between 05 days and 17 years. The symptoms and clinical signs most commonly presented by the animals were characterized by apathy, lack of appetite, progressive weight loss, nausea, prostration, fever, vomiting, severe abdominal pain, gastritis, gastroenteritis, diarrhea with blackened stools, pneumonia, anemia, leukopenia, neutrophilia, thrombocytopenia, corticosteroid blindness, paresis of hind and lower limbs, cerebellar ataxia, staggering gait, pedaling movements, convulsions, mental confusion, muscle spasms or myoclonus, nystagmus, tremors, salivation, loss of consciousness, tetany, liver with bleeding areas, alopecic wounds, hyperkeratosis of the paws, nutritional and vitamin deficiency, infestation by ecto and endoparasites (isospora, giardia, hookworm and enterobius) and death.

Samples were processed for transmission electron microscopy by negative staining (rapid preparation) and resin embedding techniques and for histopathology by hematoxylin and eosin (H&E) technique.

2.2 Transmission Electron Microscopy

2.2.1 Negative staining technique (rapid preparation)

In this technique, stool, urine, swabs nasal e ocular and cérebro fragments samples were suspended in phosphate buffer 0.1 M, pH 7.0. Drops of the obtained suspensions were placed in contact with metallic copper grids with carbon stabilized supporting film of 0.5% collodion in amyl acetate. Next, the grids were drained with filter paper and negatively stained at 2% ammonium molybdate, pH 5.0 (Brenner & Horne, 1959; Hayat & Miller, 1990; Madeley, 1997).

2.2.2 Resin embedding technique

Brain fragments were fixed in 2.5% glutaraldehyde in 0.1 M, pH7.0 phosphate buffer and post-fixed in 1% osmium tetroxide in the same buffer. After dehydration in cetonic series, the fragments were embedded in Spurr resin (González- Santander 1969; Luft, 1961). Ultrathin sections were cut on the LKB ultratome and mounted on copper grids. The sections were contrasted with uranyl acetate-lead citrate (Watson, 1958; Reynolds, 1963).

All grids submitted to the above reactions were observed in a Philips EM 208 electron microscope, at 80 kV.

2.3 Histopathology

2.3.1 Routine histological technique

All brain fragments were fixed in 10% buffered formalin, dehydrated, diaphanized and embedded in paraffin. 5 mm thick sections were performed and stained with hematoxylin and eosin technique.

III. RESULTS AND DISCUSSION

3.1 Clinical cases

Of the 622 samples of feces, urine, nasal and ocular swabs and brain fragments from dogs, 254 (40.83%) were positive for paramyxovirus by the negative staining technique (rapid preparation). The age of the animals ranged from 05 days to 17 years, and the percentage of positive animals up to 11 months was higher (46.8%) than in dogs aged over 1 year (28%). With reference to sex, 114 (44.88%) were positive females and 108 (42.5%) were males. In 32 samples, the sex of the animals was not identified. About 14 samples (5.51%) were co-infected with coronavirus and 11 samples (4.33%) with parvovirus. Three samples had the association of paramyxovirus, parvovirus and isospora and 3 had paramyxovirus and ehrlichiosis.

3.2 Transmission Electron Microscopy

3.2.1 Negative staining (rapid preparation) technique

During examination under the transmission electron microscope using the negative staining technique, 254 samples of feces, urine, ocular and nasal swabs and brain fragments were observed to contain paramyxovirus particles, pleomorphic, spherical (**fig. 1, big arrow**) or filamentous (**fig. 1, minor arrow**), ranging in diameter from 100-500 nm, with a outer envelope containing fine surface projections or spikes (**fig. 2, big arrow**) approximately 9 nm long. A helical nucleocapsid **with a characteristic " herring-bone" appearance** (**fig. 2 minor arrow**) com 15 nm of diameter, was also observed.

3.2.2 Resin embedding technique

The ultrathin sections of brain fragments revealed the presence of nuclei with marginalized and densely packed chromatin (**fig. 3, big arrow**), containing intranuclear inclusions (**fig. 3, minor arrow**). Intracytoplasmic granular amorphous inclusions surrounded by membrane (**figs. 4,5 (big arrow**), formed by viral nucleocapsids were also observed. Complete particles measuring 100 to 250 nm in diameter (**fig. 6, big arrow**) and incomplete particles, measuring on average 70 nm in diameter, budding from the plasma membrane could also be identified (**fig. 6, minor arrow**). Large areas of demyelination were also observed **(fig.7, arrow**).

3.3 Histopathology

3.3.1 Routine histological technique (H&E).

Under a direct light microscope in routine histopathological examination of the brain, a very marked perivascular and focal mono and polyclonal encephalitis was observed, as well a monoclonal meningitis (fig. 8, 100x). The blood vessels were congested and the parenchyma showed areas of demyelination (Fig 9, 40x). There was the presence of eosinophilic inclusion bodies in several nerve cells (corpuscles of Lentz) (fig. 10, arrow, 100x).

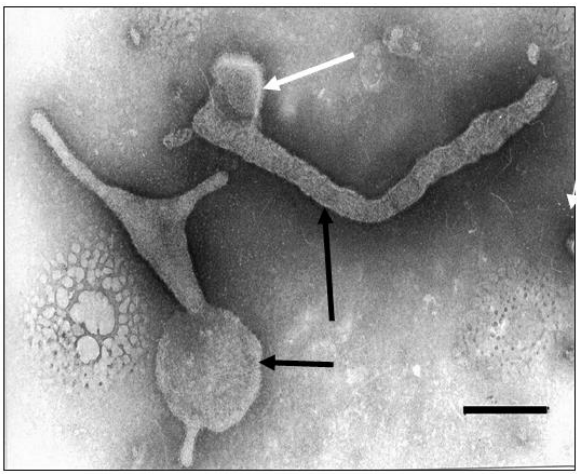

Figure 1: Negative staining of paramyxovirus in dog feces suspension, exhibiting pleomorphic and filamentous (big arrow) and rounded particles (minor arrow) and an envelope with club-shaped spikes (white arrow). Bar: 100 nm.

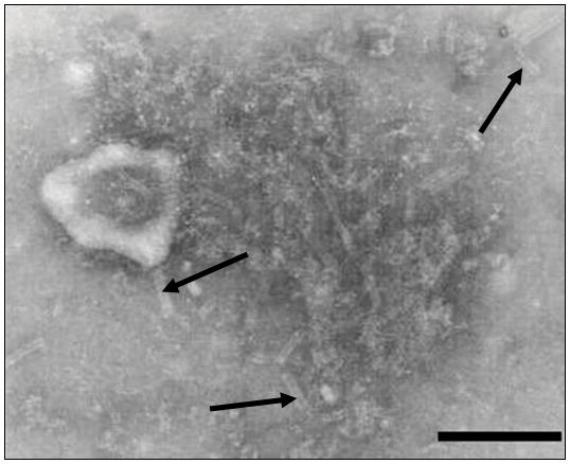

Figure 2: Negative staining of paramyxovirus particles in dog lung suspension, showing herringbone-like nucleocapsid (arrow). Bar: 180 nm.

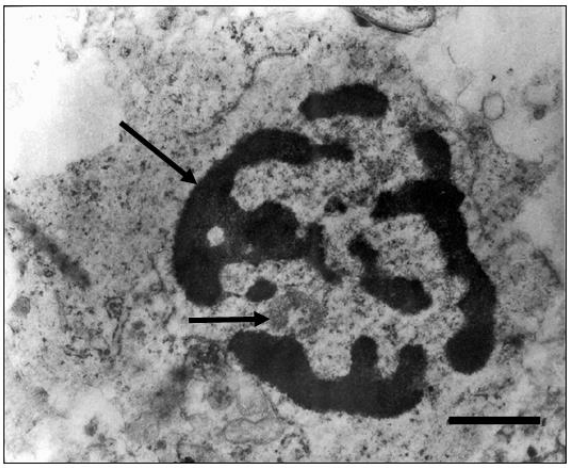

Figure 3: Ultrathin section of the brain showing a nucleus with marginalized and densely packed chromatin (big arrow) and intranuclear inclusion (minor arrow). Bar: 1600 nm.

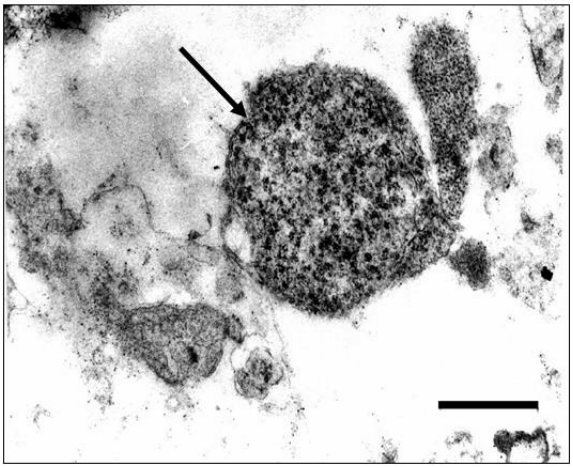

Figure 4: Ultrathin section of the brain showing Intracytoplasmic granular amorphous inclusions, formed by viral nucleocapsids (arrow). Bar: 900 nm.

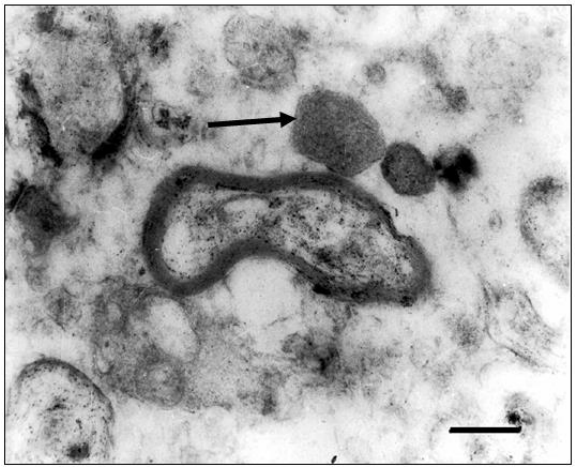

Figure 5: Ultrathin section of the brain, exhibing intracytoplasmic inclusions fomed by viral nucleocapsids (arrow). Bar: 500 nm

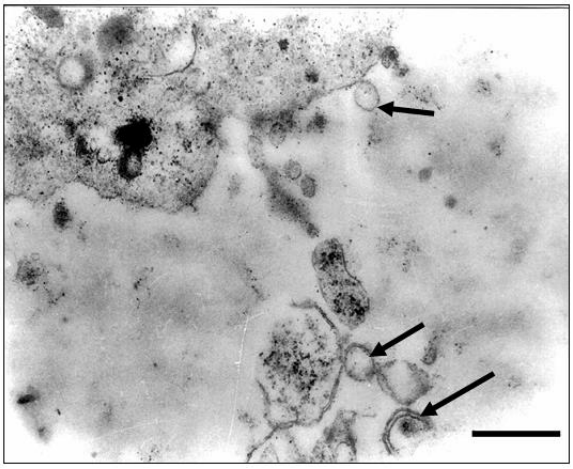

Figure 6: Ultrathin section of the brain evidencing complete particles (big arrow) and incomplete particles, budding from the plasma membrane (minor arrow). Bar: 400 nm.

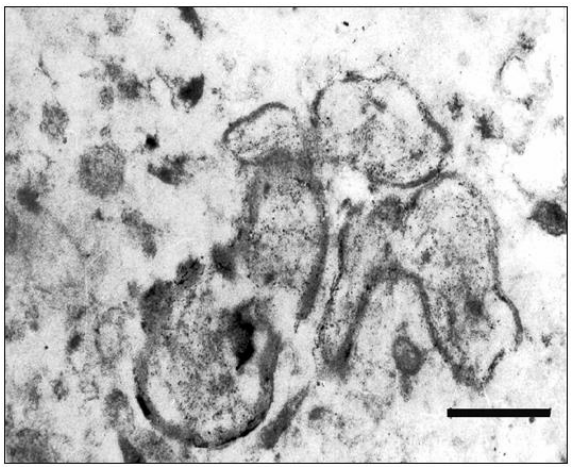

Figure 7: Ultrathin section of the brain, where large areas of demyelination are observed. Bar: 1000 nm.

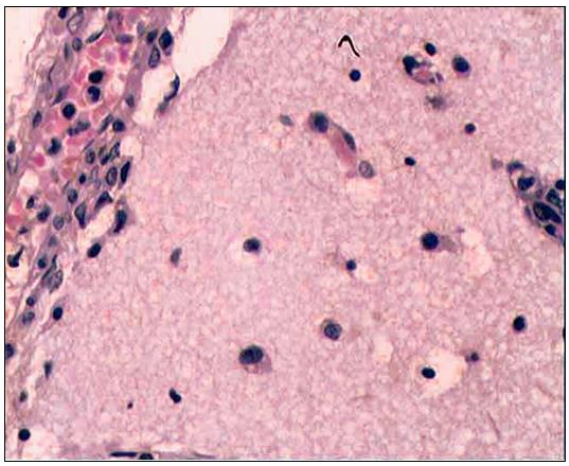

Figure 8: Histological section of the brain, where a very marked perivascular and focal mono and polyclonal encephalitis is visualized, as well a monoclonal meningitis (100x).

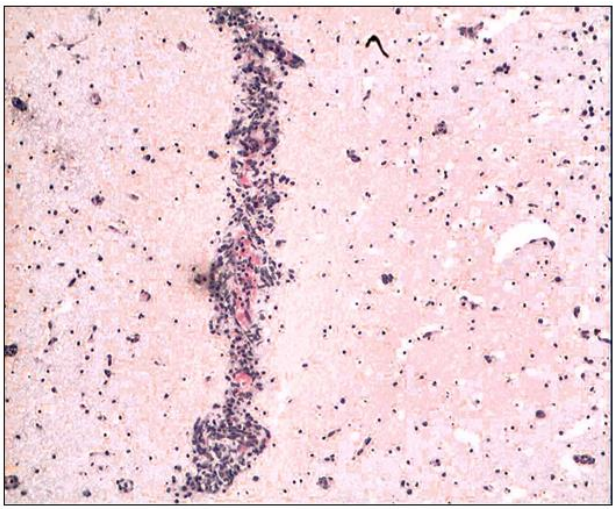

Figure 9: Histological section of the brain, showing congested blood vessels and parenchyma with areas of demyelination (40x)

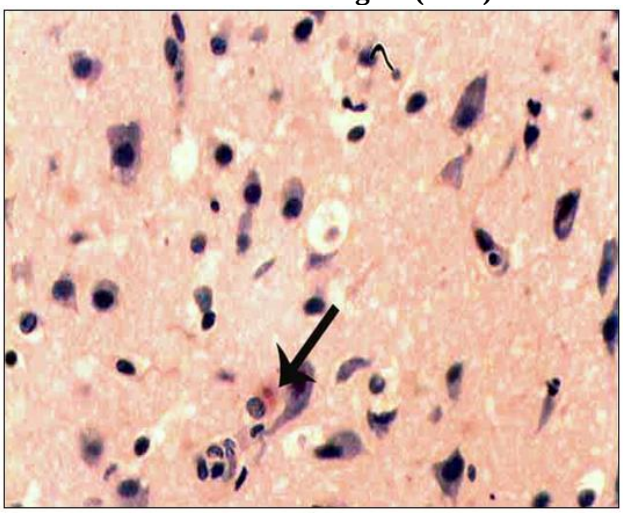

Figure 10: Histological section of the brain, showing the presence of eosinophilic inclusion bodies in several nerve cells (corpuscles of Lentz) (100x)

Transmission electron microscopy using the negative staining technique (rapid preparation) has been used for viral diagnosis, where the particles are visualized directly in the sample (Goldsmith & Miller, 2009). In this study, a total of 622 dog samples were sent to the Electron Microscopy Laboratory of the Biological Institute of São Paulo, SP, Brazil, for research of viral agents. Of these, 254 samples (40.83%) were positive for canine distemper using the negative staining technique (rapid preparation). Using this same technique associated with others, Schumaker et al. (2012) found 100% positivity during an outbreak of canine distemper that occurred in the USA and Li et al. (2013) reported about 96% in China. In other studies, on canine distemper, positivity showed to be quite oscillating, varying between 4.04 and 7.44 (Sarchahi et al., 2022) and 85% in Iran (Mousafarkhani et al., 2023); 6.9 and 29.62% in Iraq (Mohammad et al., 2022); 11.3% in the Himalayan (Dorji et al., 2020); 18% in India (Pranitha et al., 2022) and 60.9% in Portugal (São João et al., 2021). In Italy, Alfano et al. (2022) reported 18.4% in imported dogs, 74% in stray dogs and 3.9% in domestic dogs. In Brazil, positivity also varied between 2.63% (Santos et al., 2020); 34% (Costa et al., 2019) and 68.75% (Jesus et al., 2021). Menezes et al. (2023) described 82.61% mortality rate. Other authors, however, detected the presence of the virus in asymptomatic dogs (Del Puerto et al., 2010) and in healthy dogs (Mohammad et al., 2022).

Some of the different symptoms and clinical signs commonly presented by the animals in our study were also mentioned in other studies on distemper in dogs (Amude et al., 2006; Gowtage-Sequeira et al., 2009; Del Puerto et al., 2010; Schumaker et al., 2012; Li et al., 2013; Santos et al., 2020; Jesus et al., 2021; Johnson et al., 2021; São João et al., 2021; Silva et al., 2022; Menezes et al., 2023 and Mousafarkhani et al., 2023). Other symptoms have also been referenced, such as paralysis of the

mandible, urinary bladder and rectum (Catroxo et al., 2003), enamel hypoplasia, aggressiveness, vocalization, restlessness and palmigrade postures (Menezes et al., 2023), renal abnormalities in terminal patients (Silva et al., 2022) and metaphyseal sclerosis secondary to canine distemper virus (Johnson et al., 2022).

With regard to age, the dogs in our research ranged from 05 days to 17 years, and the percentage of animals infected up to 11 months was higher (46.8%) than in dogs aged over 1 year (28.0 %). Corroborating our data, other authors reported that 70% of the animals in their study were younger than 12 months (Mousafarkhani et al., 2022), higher positivity in puppies 8-14 weeks old (Schumaker et al., 2012), with less than 6 months (Braund, 1994) or that most were puppies (Alfano et al., 2022). Contrary to other studies, a higher incidence of the disease has been reported in animals aged over 12 months (19.04%) (Pranitha et al., 2022), over 3 years (22.73%) (Mohammad et al., 2022), between 1 and 8 years (Jesus et al., 2021) and 1 and 6 years (41.30%) (Menezes et al., 2023). Headley & Graça (2000) stated that the age of the animals most affected by CDV they studied was from 0 to 1.5 year and Silva et al., (2022) from 4 months to 2 years.

With regard to gender, we found 44.88% positivity in females and 42.51% in males, with no significant differences between them, which was also confirmed by Dorji et al. (2020), São João et al. (2021) and Menezes et al. (2023). Other studies, however, reported a higher percentage in infected female dogs (25.23%) (Mohammad et al., 2022) and 71.42% and 20% in males, respectively (Jesus et al., 2021; Pranitha et al., 2022). Mousafarkhani et al. (2022) mentioned that the female dogs in their study were more susceptible than male dogs, but the mortality rate from male dogs was higher than that of female dogs.

Fourteen animals in our study (5.51%) were coinfected with coronavirus and 11 (4.33%) with parvovirus. Zhao et al. (2016) reported a 1.11% rate of co-infection with coronavirus and 4.7% with parvovirus in dogs in their study, while Deng et al. (2023) reported 26.7% coinfection with coronavirus and Headley et al. (2018) 100% with parvovirus. Stilwell et al. (2019) reported dual infection with emergent strain of canine distemper virus and canine parvovirus in an Arctic wolf (*Canis lupus arctos*). Three animals were co-infected with *Erlichia canis*, a fact also reported in a young female during research by Santos et al. (2020). Considering that the canine distemper virus causes immunosuppression, concomitant infections may arise, as is the case with *Erlichia canis*, hindering the organism response to other parasites, which leads to aggravation of the clinical condition (Schneider et al., 2017).

Likewise, three other dogs had the association of paramyxovirus, parvovirus and isospora. Isosporosis has been associated with diarrhea, infecting young dogs subjected to stress factors, which leads to immunocompetence of the immune system (Rodrigues and Menezes, 2003) and predisposing animals to viral diseases that have tropism for the intestinal and respiratory epithelium and nervous system, as is the case with distemper virus, parvovirus and coronavirus (Silva et al., 2007). These multiple viral infections occur frequently and contribute to sudden death in puppies. Canine infectious morbillivirus continues to be one of the most important disease agents of puppies and due to its immunosuppressive effects can facilitate the development of other infectious disease pathogens (Headley et al., 2018).

Paramyxovirus particles, pleomorphic, rounded or elongated, measuring 100 to 500 nm in diameter, containing an envelope covered by spikes and helical herring-bone-like nucleocapsid, were visualized in all 254 positive samples from dogs, using the negative staining technique, under transmission electron microscope. These morphological aspects were also described by other authors in other research on distemper in dogs (Schulz et al., 2008; Woma et al., 2009; Tan et al., 2011; Schumaker et al., 2012; Li et al., 2013; Yang et al., 2020), in ferrets (Williams et al., 1988; Catroxo et al., 2010), in rhesus monkeys (Sun et al., 2009), and in a raccoon dog (Cheng et al., 2015). In ultrathin sections of brain fragments, intracytoplasmic inclusions formed by viral nucleocapsids were visualized, also observed in other investigations of canine distemper, in heart muscle cells (Higgins et al., 1981), in neutrophil cells (Mc Laughlin et al., 1985) , in adipose stem cell (Altamirano-Samaniezo et al., 2022), within the bronchiolar epithelial cells of the lions, tigers, and leopards (Appel et al., 1994), and, in pulmonary syncytial cells in dolphin (*Tursiops truncatus*) (Stone et al., 2011). Ultrathin sections revealed the frequency of intranuclear inclusions, as also reported by Chludzinski et al. (2023) and by Stone et al. (2011). The appearance of the nucleus with marginalized and densely packed chromatin that we visualized was observed by Altamiro-Samaniezo et al. (2022). The presence of complete particles measuring 100 to 250 nm in diameter and incomplete particles, measuring on average 70 nm in diameter, budding from the plasma membrane that we identified was not visualized in cardiac muscle cells from dogs infected with distemper, in a study carried out by Higgins et al. (1981). These ultrastructural aspects, however, were reported in another study of avian paramyxoviruses (Catroxo et al., 2023). According to Goldsmith & Miller (2009) paramyxoviruses can obtain their outer membrane by budding into cytoplasmic vesicles or out of the plasma membrane.

In the histopathological examination of the brain, a very marked perivascular and focal mono and polyclonal encephalitis was observed, as well a monoclonal meningitis, which were also visualized in other histopathological studies of distemper in dogs

(Headley & Graça, 2000; Johnson et al., 2022; Higgins et al., 1981), in lions, tigers and leopards (Appel et al., 1994); in jackals (Goutage-Sequeira et al., 2009); in dolphin (Stone et al., 2011), and in ferret (Williams et al., 1988). The presence of eosinophilic inclusion bodies (Lentz bodies), which we found in several nerve cells, was also reported by other authors, in dogs (Headley & Graça, 2000); in arctic wolf (Stilwell et al., 2019); in lions, tigers and leopards (Appel et al., 1994); in jackls (Goutage-Sequeira et al., 2009); in dolphin (Stone et al., 2011) and in ferret (Williams et al., 1988), being considered pathognomonic in the diagnosis of canine distemper (Thrall, 2015). Other authors found these Lentz inclusions in blood cells (Leal et al., 2011; Schneider et al., 2017; Castillo et al., 2019); skin (Areco et al., 2022), corneal epithelium (Headley et al., 2018), in osteoclasts (Johnson et al., 2022), and, in bronchial epithelial cells (Tamukai et al., 2020).

The techniques used were useful for rapid diagnosis, since they detected the viral presence directly in the samples, which allowed the immediate initiation of prophylactic measures and disease control when necessary. The different types of biological samples, nasal and oral swabs, urine, feces and organ fragments were adequate for the visualization of paramyxoviruses.

There is no specific treatment for canine distemper, but supportive therapies (fluid therapy, antibiotic therapy, use of vitamins, immunostimulants, anticonvulsants, antiemetics and analgesics) and complementary therapies (acupuncture, physiotherapy, hydrotherapy and application of stem cells) have been used to the decrease in symptoms (Dornelles et al., 2015; Greene & Vandevelde, 2015). Considering that CDV constitutes a danger both for animals in kennels, as well as for those belonging to private owners and wild carnivores, adequate vaccination prevents the spread and transmission of the disease, in addition to contributing to the preservation of wild species, avoiding events of spillover (Headley & Graça, 2000; Kapil & Yeary, 2011).

IV. CONCLUSIONS

Considering that canine distemper is a highly contagious disease that mainly affects dogs of all ages, causing a high socioeconomic impact in the Brazilian veterinary, in addition to being classified as an emerging disease also affecting vulnerable wild canids, the application of transmission electron microscopy and histopathology techniques in routine or during outbreaks of the disease may help to develop measures for prevention and control of canine distemper, in addition to assisting in the preservation of wild carnivorous species.

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Promising Eucalyptus Clones for Vindhyan Region of Uttar Pradesh

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Abstract**—** *As a short rotation and fast growing nature, Eucalyptus is widely preferred by farmers for pulp paper and plywood industries as well as in local market for poles. In eastern Uttar Pradesh, eucalypts are in improving stage for adoption at larger level and choice of suitable clones in plantations is still a big challenge for them. Thus, the main objective of this study was to assess the growth performance of Eucalyptus clones in Vindhyan region of Mirzapur district for identification of promising clones. An experimental trial was established in the year 2016 with 19 commercial clones at spacing of 2x3 meter in randomized block design in Mirzapur. The annual increment of each clone was calculated using all the growth parameters (girth at breast height; gbh and height) for consecutive five years. The highest value of increment in gbh belonged to clones P-32 (53.88 cm) followed by 07 (51.96 cm), P-14 (50.40 cm), 288 (48.24 cm), P-23 (46.89 cm), P-50 (45.79 cm), and P-13 (44.99 cm) after five year of planting. The clones with good annual increment in height were 07 (17.50 m) followed by P-32 (17.33 m). On the basis of growth parameters, viz. height, girth at breast height, basal area and tree volume, clone P-32, 07, P-14, 288, P-23, P-50 and P-13 were promising over others. (Fig.1). All superior clones belonged to species E. camaldulensis except 288 and 07 which were of E. tereticornis. The results of the analysis of variance (ANOVA) for mean height and girth increments showed high levels of significance.*

Keywords— Eucalyptus, pulp paper, plywood industries, E. tereticornis, E. camaldulensis.

I. INTRODUCTION

The early introduction of *E. camaldulensis* and *E. tereticornis* to India was from southern temperate localities in Australia rather than the northern tropical regions where the climatic conditions closely resemble the areas available in India because of the inaccessibility and difficulties in collecting seeds (Boland *et al*. 1981). Therefore, there is an urgent need for improvement in production of forest resources to meet the needs of fuel-wood, timber and wood production on a sustainable basis and increase biomass yield from farm forestry plantations (Patil *et al*. 2012, Srivastav *et al.* 2018). India has ~10% of the world's Eucalyptus plantation. As per the Food & Agriculture Organisation (FAO) Report (FP/48/E) 2014, around 93% of industrial wood requirement in the country is met out of agro/farm forestry plantations (~70% is Eucalyptus). In eastern Gangetic Plain region of Uttar Pradesh state of India, Eucalypts are in improving stage for adoption at larger level and choice of suitable clones in plantations is still a big challenge. Large scale Eucalyptus plantations have been raised on forest & farm lands, community lands and road / rail / canal strips in India. These plantations have created very useful resource for timber, poles, pulpwood and fuel-wood. However, most of these past plantations had very large genetic variation, low productivity ranging from 6 to 10 m3.ha-1.yr-1 and poor returns because inferior seed used for raising most of the target oriented plantations (Lal, 1993). As a short rotation and fast growing nature, Eucalyptus is widely preferred by farmers for pulp and paper industries as well as in local market for pole (Behera, 2016). In eastern part of Uttar Pradesh state of India, Eucalypts are in improving stage for adoption at larger level and choice of suitable clones in plantations is still a big challenge for them. Thus, the main objective of this study was to assess the growth performance of Eucalyptus clones in Vindhyan region of eastern Uttar Pradesh in Mirzapur district for identification of promising planting material.

II. MATERIAL AND METHODS

Study area - Mirzapur district is bounded on the north by Bhadohi and Varanasi districts, on the east by Chandauli district, on the south by Sonbhadra district and on the northwest by Prayagraj in eastern UP. The district occupies an area of 4521 km^2. Mirzapur city is the district headquarters. Mirzapur district is a part of Mirzapur division (Fig.1). It has an average sea elevation of 80 m (265 feet). The District of Mirzapur lies between the parallels of 23.52 & 25.32 North latitude and 82.7 and 83.33 East longitude. On the north and north-east it is bounded by the Varanasi district; on the south bounded by Sonbhadra district; on the north-west by Prayagraj district. The shape to the north and west is totally regular. In no direction, except for

about 13 km in the north-east where the Ganges separates the Tehsil of Chunar from the district of Varanasi, has Mirzapur a natural frontier. According to the Central Statistical Organisation, the district of Mirzapur had an area of 4521 km^2 (Censusindia.gov.in, 2011).

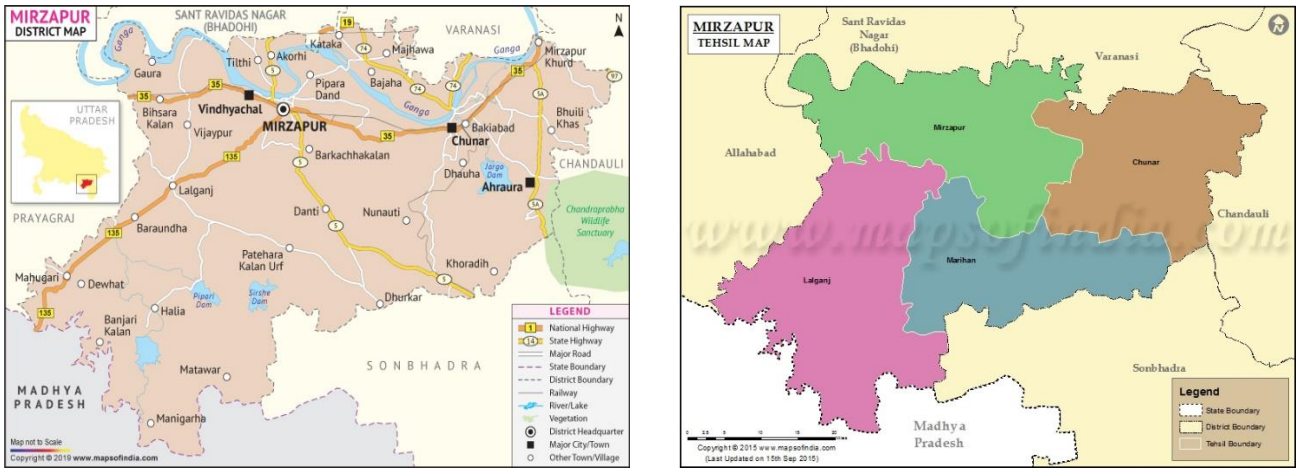

FIGURE 1: Map of district Mirzapur

III. ESTABLISHMENT OF EXPERIMENTAL TRIAL

An experimental trial was established in year 2016 with 19 commercial clones at spacing of 2x3 meter in village Nikarika. It is a small Village/hamlet in Raigarh Block/tehsil of Mirzapur District of Uttar Pradesh State and located at a distance of 55 km from district head quarter. A total of 20 treatments with 19 clones and one control in randomized block design on field bunds in three replications were taken under study. The soil analysis was done for pH, EC, organic carbon and NPK contents using standard procedures (Jackson, 1985). The mixture of 100 g of NPK (3:2:1) fertilizer and FYM (1.0 kg per plant) were applied at onset of monsoon during planting to assist establishment of growth. The irrigation was also done twice a month normally and in hot summers once in a week. The annual increment of each clone was calculated using all the growth parameters (girth at breast height; gbh and height) for consecutive 4 years. The basal area in m^2 (BA =0.00007854 x DBH in cm) and volume of trees in cum ($V= \pi r^2 x h$) / tree (r and h in m), (1667 trees / ha in 3x2 spacing) were also calculated (Larsen, 1999). The annual increment of each clone was calculated using all the growth parameters for girth at breast height and height after one year of planting to assess early growth performance of clones. The data were statistically analysed by standard ANOVA techniques (XLSTAT). The statistical analysis was done by data analysis tool package of OPSTAT prepared by Statistical Software Package for Agricultural Research Workers. CCS HAU, Hisar, Haryana (Sheoran *et al*. 1998). The details of clones were as following:

THE DETAILS OF CLONES AND SPECIES NAME

S. No.	Clone No	Species name
1.	P13	*E. camaldulensis*
2.	2136	*E. camaldulensis*
3.	P50	*E. camaldulensis*
4.	P23	*E. camaldulensis*
5.	526	*E. camaldulensis*
6.	P66	*E. camaldulensis*
7.	2070	*E. camaldulensis*
8.	288	*E. tereticornis*
9.	2023	*E. camaldulensis*
10.	P32	*E. camaldulensis*
11.	413	*E. camaldulensis*
12.	P14	*E. camaldulensis*
13.	3018	*E. hybrid*
14.	K25	*E. camaldulensis*
15.	2021	*E. camaldulensis*
16.	07	*E. tereticornis*
17.	P45	*E. camaldulensis*
18.	2013	*E. camaldulensis*
19.	04	*E. camaldulensis*

IV. RESULTS AND DISCUSSION

The results of growth performance of these clones were recorded for annual increments of height (m) and girth at breast height; gbh (cm) for five years and were depicted in Table 1& 2 and Fig. 2 & 3. The site was with red acidic soil and analysis indicated pH 6.10, EC 0.42 mm/cm, organic carbon 0.21 %, Nitrogen 182.44 kg/ha, Phosphorus 11.23 kg/ha and Potassium 220.38 kg/ha. The highest value of gbh belonged to clones P-32(53.88 cm) followed by 07 (51.96 cm), P-14 (50.40 cm), 288 (48.24 cm), P-23 (46.89 cm), P-50 (45.79 cm), and P-13 (44.99 cm) after five year of planting. The lowest values belonged to clone 2013 and P-66 with 38.58 and 40.82 cm respectively. The clones with good increment in height were 07 (17.50 m) followed by P-32 (17.33 m). The basal area and tree volume were also analysed.On the basis of growth parameters, *viz.* height, girth at breast height, basal area and tree volume, clones P-32, 07, P-14, 288, P-23, P-50 and P-13 performed superior over all other treatments in Mirzapur district (Table 3, Fig. 4, 5 & 6). All superior clones belonged to species *E. camaldulensis* except 288 and 07 which were of *E. tereticornis.* The results of the analysis of variance (ANOVA) for mean height and girth increments showed high levels of significance. The results of growth performance indicated that all clones gave superior results for growth indicators as compared to control. The performance of control was inferior for girth increments as compared to clone series. The remaining clones had different ranks of gbh and height increments as compared to the control.

TABLE 1
ANNUAL INCREMENT IN HEIGHT (M) IN MIRZAPUR IN 4 YRS

S. No.	Clone	Year 1	Year 2	Year 3	Year 4
1	P66	2.24	2.46	4.06	3.11
2	K25	2.90	1.69	5.29	4.24
3	07	2.79	4.40	8.14	1.87
4	P32	3.84	5.11	6.77	2.16
5	04	3.47	5.81	5.67	2.99
6	3021	2.64	4.84	3.41	6.28
7	2070	2.83	3.43	5.39	5.30
8	413	2.19	2.75	6.08	4.81
9	2023	2.19	2.40	2.87	7.52
10	526	2.12	4.11	3.61	4.82
11	2013	2.29	1.60	5.69	3.16
12	Control	1.27	1.08	1.63	5.44
13	P23	2.42	2.14	6.71	3.59
14	288	3.06	2.40	6.40	1.88
15	3018	3.20	2.82	4.64	3.35
16	2136	2.39	1.61	5.65	3.94
17	P14	2.33	2.69	5.29	4.60
18	P13	3.50	2.86	4.04	2.90
19	P50	2.70	2.92	4.27	3.34
20	P45	2.69	1.95	4.40	3.27
	C.D.	0.09	0.65	1.33	2.989
	SE(m)	0.03	0.23	0.46	1.040
	SE(d)	0.04	0.32	0.66	1.471
	C.V.	13.34	13.07	13.76	16.567

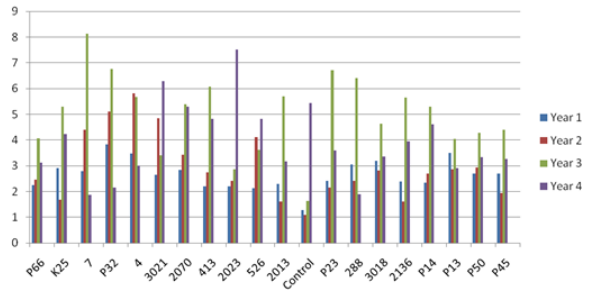

FIGURE 2: Annual increment in height (m) in 4 years

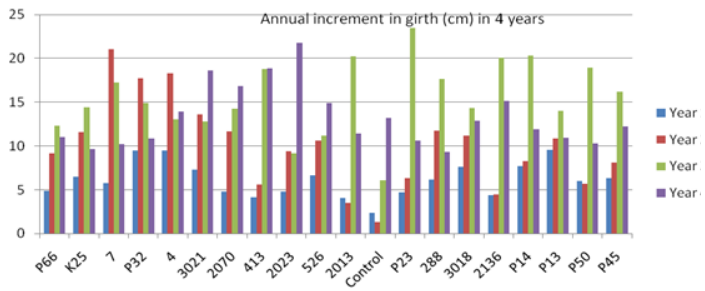

FIGURE 3. Annual increment in girth (cm) in 4 years

TABLE 2
ANNUAL INCREMENT IN GIRTH (CM) IN 4 YEARS

S. No.	Clones	Year 1	Year 2	Year 3	Year 4
1	P66	4.86	9.17	12.33	11.00
2	K25	6.50	11.57	14.43	9.64
3	07	5.72	21.07	17.23	10.20
4	P32	9.46	17.73	14.88	10.89
5	04	9.46	18.33	13.02	13.96
6	3021	7.33	13.57	12.79	18.64
7	2070	4.82	11.67	14.23	16.83
8	413	4.16	5.57	18.79	18.89
9	2023	4.80	9.37	9.16	21.80
10	526	6.62	10.60	11.19	14.91
11	2013	4.07	3.53	20.21	11.40
12	Control	2.40	1.30	6.12	13.17
13	P23	4.70	6.33	23.49	10.64
14	288	6.16	11.77	17.64	9.29
15	3018	7.59	11.17	14.36	12.89
16	2136	4.41	4.50	20.10	15.14
17	P14	7.72	8.23	20.31	11.94
18	P13	9.58	10.83	14.02	10.94
19	P50	5.99	5.67	18.91	10.29
20	P45	6.36	8.07	16.17	12.23
	C.D.	0.33	2.65	4.97	7.57
	SE(m)	0.12	0.92	1.73	2.64
	SE(d)	0.16	1.30	2.45	3.73
	C.V.	21.20	22.58	17.87	14.19

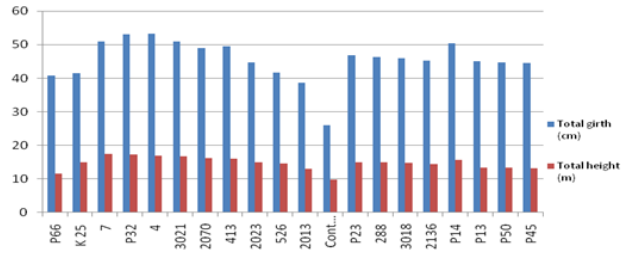

FIGURE 4: Total girth (cm) and height (m) after 4 years

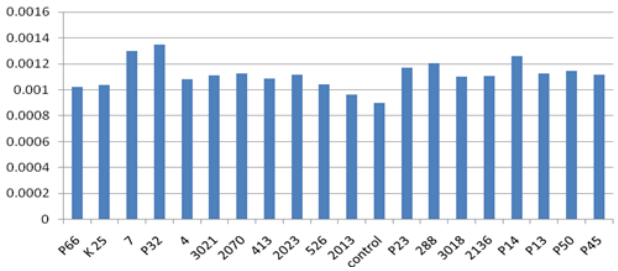

FIGURE 5: Basal area of clonal tree (m^2)

TABLE 3
GROWTH PERFORMANCE FOR BASAL AREA AND TREE VOLUME IN 4 YEAR

S.N.	Growth performance of clones after 4 years				
	Clone	Total girth including initial girth (cm)	Total height including initial height (m)	Basal Area/tree (m^2)	Volume/tree (m^3)
1	P66	40.82	11.52	0.001021	0.541539
2	K 25	41.44	14.92	0.001037	0.566591
3	07	50.96	17.50	0.001300	1.116909
4	P32	53.08	17.33	0.001348	1.245355
5	04	53.22	16.94	0.001081	0.642783
6	3021	50.93	16.78	0.001109	0.693591
7	2070	48.99	16.25	0.001125	0.725034
8	413	49.48	15.94	0.001088	0.654454
9	2023	44.67	14.86	0.001117	0.709673
10	526	41.71	14.58	0.001043	0.577738
11	2013	38.58	12.90	0.000965	0.457190
12	Control	26.03	9.85	0.000901	0.372394
13	P23	46.89	14.93	0.001173	0.820826
14	288	46.24	14.97	0.001207	0.893783
15	3018	45.86	14.76	0.001102	0.680995
16	2136	45.28	14.33	0.001108	0.691247
17	P14	50.40	15.70	0.001261	1.019300
18	P13	44.99	13.36	0.001125	0.725034
19	P50	44.79	13.26	0.001145	0.764403
20	P45	44.54	13.22	0.001114	0.703495
	C.D.	7.38	2.10	-	-
	SE(m)	2.57	0.73	-	-
	SE(d)	3.63	1.03	-	-
	C.V.	9.84	8.60	-	-

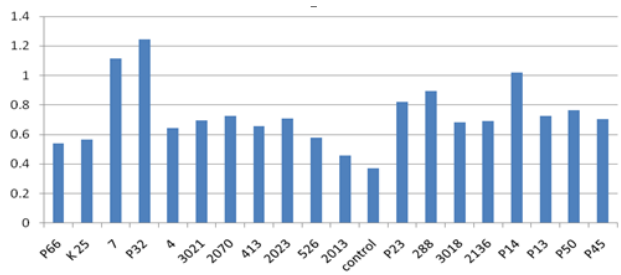

FIGURE 6: Volume of clonal tree (m^3)

Luna and Singh (2009) studied the growth performance of 12 clones of Eucalyptus at Ludhiana. Clones 413 and 2070 recorded significantly higher height growth as compared to other clones. In south Gujrat, clonal variation for growth parameters such as DBH, mid- diameter, height, form quotient and volume was significantly different among 20 clones of Eucalyptus and DBH varied between 11.47 and 16.07 cm with an overall mean of 13.28 cm (Bahera, 2016). Kumar *et al* (2006) evaluated different clones of *Eucalyptus tereticornis* for different growth characters including tree height. Gangwar *et al.* (2015) identified Clone AP10 of Eucalyptus as the best clone among the studied ones based on height. It is also established that *E. camaldulensis* as a pure species is adapted to low-to intermediate rainfall environment with a dry season of up to 8 months (Eldridge *et al.* 1993).

Lal *et al.* (2005) evaluated growth performance of 36 clones of Eucalyptus and clone 2070 performed best with maximum mean height of 16.29 m as compared to other clones at the age of 6 years. The variation among clones in growth parameter may be due to genetic make-up and interactions with the environmental factors. Similarly, Kumar *et al.* (2006) reported significant variation among clones of *E. tereticornis* for DBH at the age of 5.5 years, whereas Dhillon and Singh (2010) also found difference in diameter growth among clones of *E. tereticornis* at the age of 3.5 years. Lal *et al* (2006) identified best clones out of 36 *viz.*, clone 2070, 285, 316, 288, 498, 286 and 2045 for Punjab ecological condition.

Wei Zhongmian *et al.*, (2009) evaluated the growth comparison of eight year old Eucalyptus clones revealed that, Eucalyptus clones showed high growth rate during the first three years. There were significant differences amongst the 3 Eucalyptus clones in plant height, diameter and volume growth. The significant G x E interaction observed among clones/seed origin on test site in the present study indicates that clones have to be tested in target environments before deploying in plantations (Oballa *et al*., 2005).

Among the 22 clones of *Eucalyptus camaldulensis* planted in the clonal trial at Badami (Karnataka) clones Clone C- 10,19,188 are performing better than all other clone planted in that particular location having same soil property (sandy loam) and climatic conditions (Vijayaraghavan et al., 2015). The fact that most clones outperformed the provenance seed lots at comparatively waterlogged condition (Karaikkal); whereas some clones were inferior to the best provenance seed lot demonstrates that clonal selections should not be transferred to contrasting environments without thorough testing (Vijayaraghavan et al., 2016). The clones of species *E. camaldulensis* performed superior over other clones/species. Similarly, significant differences in different *Eucalyptus* species have been reported by various workers.

The variation among clones in growth parameter may be due to genetic make-up and interactions with the environmental factors. Similarly, Dhillon and Singh (2010) also found difference in diameter growth among clones of *E. tereticornis* at the age of 3.5 years. Lal *et al* (2006) identified best clones out of 36 *viz*., clone 2070, 285, 316, 288, 498, 286 and 2045 for Punjab ecological condition. Red Gum (*Eucalyptus camaldulensis* L.) is renowned globally for its fast growth, high levels of drought tolerance and adaptability to diverse climatic conditions and soils, which makes it popular among eucalypt tree growers (Bindumadhava *et al*. 2011). The results of study confirm that clones of *E. camaldulensis* are well adaptable in Vindhyan region of Mirzapur district of Eastern Uttar Pradesh in India.

V. CONCLUSION

It is clear from the study that selection of clones for a particular site is very important to get maximum productivity of clonal eucalypts plantations in and around Eastern Uttar Pradesh. In addition, this study demonstrated that there would be clear benefits, with respect to productivity of a large eucalypt plantation to pursuing site-specific selection and deployment strategies for the high productive clones. Although implementing such a strategy could require significant investments in field trials, for larger growers with plantations spread across site types, the benefits with respect to increased clonal plantation with site specific clones would be more beneficial. Thus, suitable clones of eucalypts may improve agroforestry in the region of eastern UP. The identified commercial clones of Eucalypts may open a new path for stakeholders of the region for more adoption of species in agroforestry models with better returns after shorter duration of time. Therefore, clonal plantations of Eucalyptus under agro-forestry system should be encouraged and integrated with planned development of wood based industries through innovative policy changes.

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An Analysis on the Effect of Atonik Plant Growth Regulator on the Growth and Yield of Several Early Maturing Soybean (Glycine Max L.) Genotype

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Abstract— *This research aims to determine the effect of Atonik plant growth regulator (PGR) on the growth and yield of early maturing soybean (Glycine max L.) genotype. The study was conducted as a field experiment using polybags and employed a split plot design (SPD) with two factors. The main factor was four concentrations of Atonik PGR (Z), namely ZO (0 ml/l), Z1 (0.5 ml/l water), Z2 (1 ml/l water), and Z3 (1.5 ml/l water). The sub-factor was the soybean genotypes (G), which were G1 (GH 8 soybean line), G2 (GH 63 soybean line), G3 (GH 73 P soybean line), and G4 (Dega 1 soybean variety). The combinations of treatments were replicated three times. The observed variables included the number of leaves, the number of productive branches, the number of seeds each plant, the weight of seeds each plant, and the weight of 100 seeds. The research findings showed that the concentration of Atonik PGR at 1 ml/l water resulted in the highest number of productive branches, which is 5.25 branches, the highest number of seeds each plant, which is 55.14 seeds, and the heaviest weight of seeds each plant, which is 9.34 g. The concentration of Atonik PGR at 1.5 ml/l water produced the heaviest weight of 100 seeds, which is 23.21 g. The GH 63 soybean line produced the highest number of leaves each plant, which is 92.77 leaves, and the highest number of productive branches each plant, which is 5.25 branches. The GH 8 soybean line produced the highest number of seeds each plant, 62.16 seeds, and the heaviest weight of 100 seeds each plant, 20.49 g. The Dega 1 soybean variety produced the heaviest weight of seeds each plant, which is 9.90 g.*

Keywords— Soybean (Glycine max L.), PGR, GH 8, GH 63, GH 73 P, Dega 1.

I. INTRODUCTION

Soybean (*Glycine max* (L) Merril) is an essential commodity in agriculture, particularly as a source of raw materials for industries, feed, and food. Soybeans are a food source with numerous benefits, providing plant-based protein and fats, while soybean oil produces vitamin E (Sunarto, 2000). Soybean is a significant commodity alongside rice and corn due to its high nutritional content, mainly plant-based protein (Jusniati, 2013).

The demand for soybeans in Indonesia is high, but its supply needs to be increased due to low production, leading to heavy reliance on imports. Low cultivation technology, reduced planting areas, cheap imported soybeans, and prolonged dry seasons have contributed to low domestic soybean production (Rahmasari et al., 2016).

The increasing population in Indonesia has resulted in a higher demand for soybeans (Permadi, 2015). One way to optimize soybean productivity is by using plant growth regulators (PGRs). PGRs are non-nutritional organic compounds that are active

in optimal concentrations to stimulate, inhibit, or modify plant growth and development. The use of PGRs aims to control plant growth. Some commonly used PGRs are relatively expensive and difficult to obtain. Thus, alternative synthetic PGRs are needed (Lestari, 2011).

Atonik is a liquid plant growth regulator (PGR) with a brownish color, belonging to the group of auxins. It contains active ingredients such as triacontanol, sodium phenolic (Na-Ortonitrophenol 0.2%, (C6H4NO3Na) 0.3%), Na-paranitrophenol (CP6H4NO3Na) 0.1%, Na-5 nitroquaniakol (C7H6NO3Na), and 0.5% Na-2,4 dinitrophenol (CP6H3N2O5Na). The Na+ ion functions as a carrier of metabolites in the metabolic process and can partially replace the function of the K+ ion. The primary function of Atonik is to stimulate plant growth (Ritonga, 2020).

Several studies have shown that applying plant growth regulators (PGRs) through leaves enhances plant growth and yield compared to soil application (Hanolo, 1997). This is because the use of Atonik PGR can affect the number of plant leaves. Atonik is a synthetic growth-promoting substance that stimulates root growth, activates nutrient uptake, increases bud emergence, and improves plant quality (Trisna, 2013).

Ismail and Effendi (1985) stated that early-maturing soybeans benefit farmers as they allow crop rotation with rice and help avoid water shortages during soybean growth. Furthermore, early-maturing soybeans can provide advantages such as reducing pest attacks and increasing the crop index yearly. In some soybean production centers, early-maturing varieties with larger seed sizes are preferred by farmers (Krisnawati and Adie, 2007).

Based on the information above, it is necessary to conduct research using Atonik PGR to analyze its effect on the growth and yield of early-maturing soybean genotypes (*Glycine max* L.).

II. MATERIALS AND METHODS

2.1 Research Location

The research was conducted at the Indonesian Legume and Tuber Crops Research Institute (BALITKABI) or Kendalpayak Village, Pakisaji District, Malang Regency. It is located at coordinates 8° 2' 56.4"LS 112° 37' 30"BT with an elevation of 445 meters above sea level. The Applied Agrotechnology Laboratory, Faculty of Agriculture and Fisheries, Universitas Muhammadiyah Purwokerto, was also used for the research. The research was conducted from September 2022 to January 2023.

2.2 Procedure

The research was a polybag field experiment and employed a split plot design (SPD) with two factors. The main factor consisted of four concentrations of Atonik PGR (Z): ZO (0 ml/l), Z1 (0.5 ml/l water), Z2 (1 ml/l water), and Z3 (1.5 ml/l water). The sub-factor was the soybean genotypes (G): G1 (GH 8 soybean line), G2 (GH 63 soybean line), G3 (GH 73 P soybean line), and G4 (Dega 1 soybean variety), with each treatment replicated three times. The observed variables included the number of leaves, the number of productive branches, the number of seeds each plant, the weight of seeds each plant, and the weight of 100 seeds.

The research procedure involved the preparation of the planting medium, which was a mixture of compost and inceptisol soil in polybags. Two soybean seeds were planted in each polybag, and the Atonik PGR was applied according to the specified concentrations. The PGR was sprayed onto the entire plant every 10 days until 60 days after planting. PGR application began 10 days after sowing (das), and the solution was sprayed on the entire plant until it dripped onto the soil.

2.3 Data Analysis

The data obtained from the observations were analyzed using the F-test at a 95% confidence level. The analysis continued with the Duncan Multiple Range Test (DMRT) at a 5% confidence level using Costat Statistical Software if significant differences were found.

III. RESULT AND DISCUSSION

TABLE 1

THE RESULTS OF THE ANALYSIS OF THE VARIABLE NUMBER OF LEAVES OF THE FOUR LINES OF SOYBEAN AND THE ADMINISTRATION OF GROWTH REGULATORS

Treatment	21 Das	28 Das	35 Das	42Das	49 Das	56 Das	63 Das	70 Das
Atonik Growth Regulatory Substances								
Atonik 0 ml/l (Z0)	12.83 a	19,10 a	28.20 a	39,16 a	55.00 a	65.89 a	74,16 a	79.47a
Atonik 0.5 ml/l (Z1)	12.58 a	19.02 a	28,16 a	39.45 a	55,64 a	68.50 a	76,29 a	80.77a
Atonik 1 ml/l (Z2)	12,18 a	19.62 a	29,18 a	40.85 a	59.02 a	70,70 a	79.33 a	83.95a
Atonik 1.5 ml/l (Z3)	12.72 a	18.83 a	29,29 a	38.77 a	58,16 a	69.52 a	76.79 a	80.08a
Soybean lines								
GH 8 (G1)	12.79 ab	19.02 a	28.43 b	40.91 b	56.91b	69.87 b	78.12 b	81.68 b
GH 63 (G2)	13.91 a	21.93 a	33,64 a	44.72 a	67,29 a	78.41 a	87.52 a	92.77 a
GH 73 P (G3)	11.43c	16.70 c	22.97 c	33.33 c	48.14c	61.33 c	68.75 c	72.95 c
Dega 1 (G4)	12.18 bc	18.91b	29.79 b	39,27 b	55,47 b	65.00 c	72.18 c	76.87 bc
DMRT 5%	1.25	1.48	2.59	3.75	4,27	4.84	5.08	5,24

Numbers followed by the same letter in the same column and treatment indicate no significant differences in the DMRT test at the 5% confidence level

3.1 The number of leaves

Table 1 shows that the concentration of PGR has no significant effect on the number of leaves at all observation ages. This suggests that the given concentration of Atonik may not have been sufficient to increase the leaf count and accelerate leaf formation, potentially due to suboptimal Atonik concentration. This aligns with the statement made by Uluputty (2015), which suggests that applying PGR in the appropriate concentration yields favorable results, but excessive concentrations might hinder plant growth.

The difference in soybean genotypes significantly affected the number of leaves at 21 DAS, 28 DAS, 35 DAS, 42 DAS, 49 DAS, 56 DAS, 63 DAS, and 70 DAS. This can be attributed to the distinct genetic characteristics of the four pure genotypes, as genes affect the traits and attributes of living organisms, including plant body shape, flower color, and fruit taste. Genes also determine metabolic capabilities, greatly impacting plant growth and development.

The difference in soybean genotypes significantly affects the number of leaves at 21 HST, 28 HST, 35 HST, 42 HST, 49 HST, 56 HST, 63 HST, and 70 HST. This is likely because the four pure genotypes have distinct genetic characteristics. Genes play a crucial role in determining the traits and characteristics of living organisms, including plants, influencing their body shape, flower color, and fruit taste. Genes also govern the metabolism's capability, thus significantly impacting plant growth and development.

The study revealed that the GH 63 (G2) soybean genotype exhibited the highest number of leaves at each observation age. At 21 days after sowing (DAS), it produced 13.91 leaves, which is 8.75% more compared to the GH 8 (G1), 21.70% more and significantly different from the GH 73 P (G3), and 14.20% more and significantly different from the Dega 1 (G4). At 28 DAS,

it produced 21.93 leaves, which is 15.30% more and not significantly different from the GH 8 (G1), 31.31% more and significantly different from the GH 73 P (G3), and 15.97% more and significantly different from the Dega 1 (G4). At 35 DAS, it produced 33.64 leaves, which is 18.33% more and significantly different from the GH 8 (G1), 46.45% more than the GH 73 P (G3), and 16.76% more than the Dega 1. At 42 DAS, it produced 44.72 leaves, which is 9.31% more and significantly different from the GH 8 (G1), 34.17% more than the GH 73 P (G3), and 16.35% more than the Dega 1. At 49 DAS, it produced 67.29 leaves, which is 18.24% more and significantly different from the GH 8 (G1) line, 39.78% more than the GH 73 P (G3), and 24.55% more than the Dega 1. At 56 DAS, it produced 78.41 leaves, which is 12.22% more and significantly different from the GH 8 (G1), 27.85% more than the GH 73 P (G3), and 21.87% more than the Dega 1. At 63 DAS, it produced 87.52 leaves, which is 12.03% more and significantly different from the GH 8 (G1) line, 27.30% more than the GH 73 P (G3), and 22.31% more compared to the Dega 1. At 70 DAS, it produced 92.77 leaves, which is 13.58% more and significantly different from the GH 8 (G1), 27.17% more than the GH 73 P (G3), and 20.68% more compared to the Dega 1.

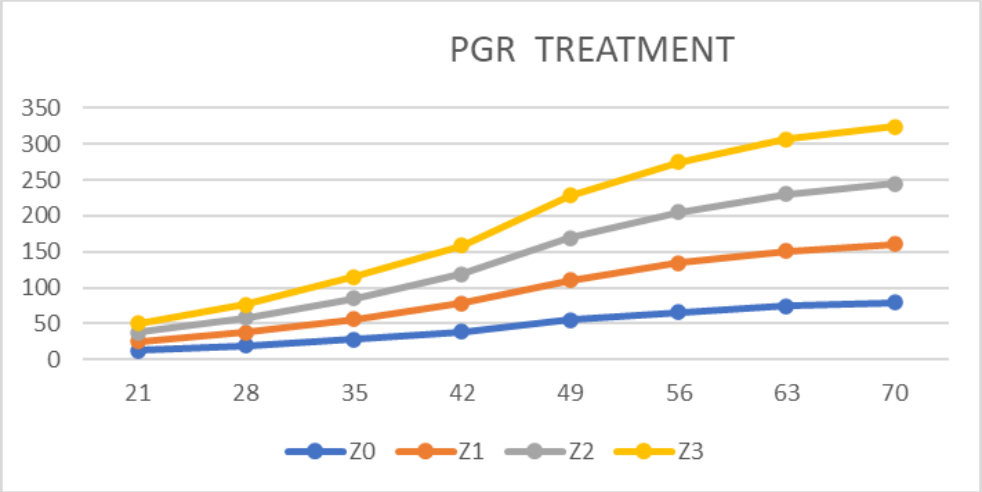

FIGURE 1: The effect of Atonik PGR concentration on the growth of the number of leaves of soybean plants

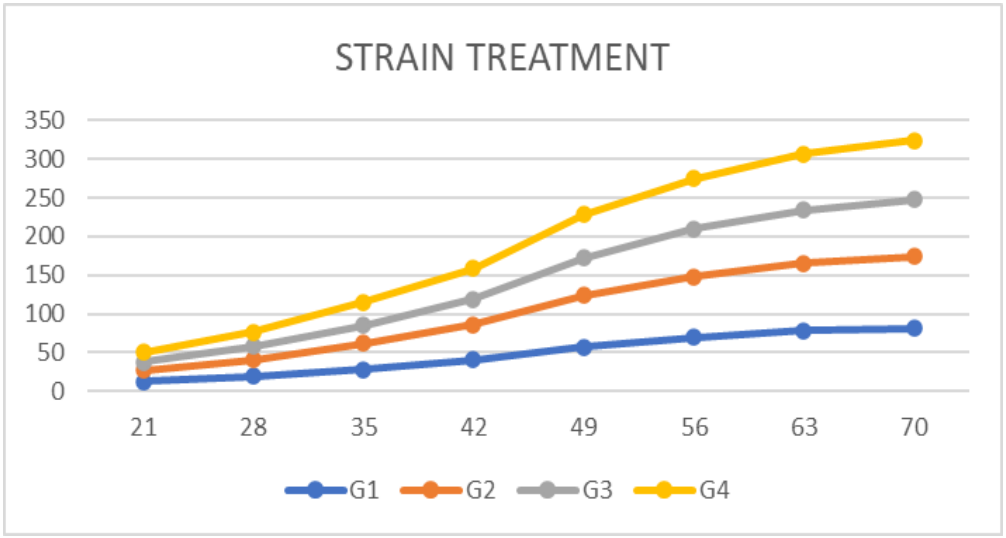

FIGURE 2: The effect of different expected ED lines on the growth of the number of leaves of soybean plants

The data illustrates that the GH 8 (G2) has the greatest number of leaves, so its photosynthetic ability is higher and will increase vegetative and generative growth. Umarie (2001) states that there are real variations and differences in all the plant characteristics observed. This is because the lines tested come from various sources and have gone through selection.

The results of data analysis on the effect of Atonik PGR and soybean lines on productive branch variables, number of seeds, seed weight, and weight of 100 seeds are presented in table 2. Table 2 shows that Atonik PGR and soybean lines significantly affect productive branches, number of seeds, seed weight, and drilled 100 seeds.

TABLE 2
THE ANALYSIS OF NUMBER OF PRODUCTIVE BRANCHES, NUMBER OF SEEDS, WEIGHT OF SEED, AND WEIGHT OF 100 SEEDS FOR FOUR SOYBEAN GENOTYPES WITH THE APPLICATION OF PLANT GROWTH REGULATOR

Treatment	Number of Productive Branches	Number of Seeds Each Plant	Seed Weight Each Plant	Weight of 100 Seeds
Atonik Growth Regulatory Substances				
Atonik 0 ml/l (Z0)	4.75 ab	52.89 a	9.08 ab	17.30 c
Atonik 0.5 ml/l (Z1)	4.68 ab	43.37 b	7.60 b	18.83 bc
Atonik 1 ml/l (Z2)	5,25 a	55.14 a	9.34 a	19.25 b
Atonik 1.5 ml/l (Z3)	4.60 b	53,52 a	8.91 ab	23,21 a
Soybean lines				
GH 8 (G1)	4.97 ab	62,16 a	9.61 ab	20,49 a
GH 63 (G2)	5.39 a	47.70 b	8.27 bc	19.65 ab
GH 73 P (G3)	4.64 bc	44.16 b	7,16 c	19.92 ab
Dega 1 (G4)	4.27 c	50.89 b	9.90 a	18.52 b
DMRT 5 %	0.58	9,15	1.50	1.80

Numbers followed by the same letter in the column and the same treatment showed no significant difference in the 5% DMRT test

3.2 The Number of Productive Branches

Table 2 shows that the concentration of Atonik plant growth regulator significantly affects the number of productive branches. This is likely because the auxin hormone present in Atonik can affect the growth of productive branches. As Pollard and Walker (1990) stated, the physiological role of auxin is to promote cell elongation, differentiation of xylem and phloem tissues, and root formation. The increase in the number of branches in plant genotypes is affected by the concentration of auxin in the region of the branch primordial (Naeem et al., 2004).

The research results show that the treatment with Atonik at a concentration of 1 ml/l (Z2) resulted in the highest number of productive branches, which is 5.25 branches. This is not significantly different from the concentration of 0 ml/l of Atonik (Z0), the concentration of 0.5 ml/l of Atonik (Z1), but significantly different from the concentration of 1.5 ml/l of Atonik (Z3). The concentration of 1 ml/l of Atonik resulted in 10.53% more productive branches compared to the concentration of 0 ml/l of Atonik (Z0), 12.18% more productive branches compared to the concentration of 0.5 ml/l of Atonik (Z1), and 13.89% more productive branches compared to the concentration of 1.5 ml/l of Atonik (Z3).

Table 2 also indicates that the difference in soybean lines significantly affects the number of productive branches. This is likely because photosynthesis results affect the varying number of branches. The GH 63 (G2) produced the highest number of leaves, producing photosynthates more. The allocation of photosynthates to the branch parts also increased, leading to the highest number of branches. The higher the number of branches in one variety, the more leaves are produced, increasing photosynthates (Sa'diyah et al., 2016).

The research results show that the GH 8 (G2) produced the highest number of productive branches, which is 5.39 branches, significantly different from the GH 73 P (G3), the Dega 1 (G4), but not significantly different from the GH 8 (G1). The GH 63 (G2) produced 8.45% more productive branches compared to the GH 8 (G1), 16.16% more productive branches compared to the GH 73 P (G3), and 24.14% more productive branches compared to the Dega 1 (G4).

3.3 Number of Seeds Each Plant

Table 2 shows that the concentration of Atonik plant growth regulator significantly affects the number of seeds each plant. This is likely because the plant quickly absorbs Atonik, stimulates protoplasmic flow in cells, and accelerates germination and rooting. However, if the concentration is excessive, it can inhibit plant growth. The optimal concentration of Atonik when sprayed through the leaves increases protein synthesis, and the synthesized proteins are used as building materials for the plant. Atonik in an optimum concentration, stimulates the growth of branches and productive nodes, thus increasing the number of

pods and seeds each plant. Atonik functions inside the plant to promote plant growth, increase yield, improve quality, and enhance crop productivity (Lestari, 2011).

The research results show that the PGR treatment with Atonik at a concentration of 1 ml/l (Z2) resulted in the highest number of seeds each plant, which is 55.14 grains. This is not significantly different from the concentration of 0 ml/l of Atonik (Z0), the concentration of 1.5 ml/l of Atonik (Z3), but significantly different from the concentration of 0.5 ml/l of Atonik (Z1). The concentration of 1 ml/l of Atonik resulted in 4.25% more seeds each plant compared to the concentration of 0 ml/l of Atonik (Z0), 27.14% more seeds compared to the concentration of 0.5 ml/l of Atonik (Z1), and 3.74% more seeds compared to the concentration of 1.5 ml/l of Atonik (Z3).

Table 2 also indicates that the difference in soybean lines significantly affects the number of seeds each plant. This is likely due to the different flowering factors and environmental conditions that support each strain, as stated by Somaatmadja (1993), indicating that flowering factors and supportive environmental conditions during pod filling determine the number of formed seeds. According to Hakim (2010), the weight of seeds each plant correlates positively with the number of seeds each plant, indicating that more leaves result in more seeds and heavier seed weight.

The research results show that the GH 8 (G1) produced the highest number of seeds each plant, which is 62.16 grains, significantly different from the GH 63 (G2), the GH 73 P (G3), and the Dega 1 (G4). The GH 8 (G1) produced 30.31% more seeds compared to the GH 63 (G2), 40.76% more seeds compared to the GH 73 P (G3), and 25.52% more seeds compared to the Dega 1 (G4).

3.4 The Weight of Seed Each Plant

Table 2 indicates that the PGR concentration of the plant growth regulator Atonik has a significant effect on the weight of seeds each plant. This is likely because Atonik stimulates the process of photosynthesis. When photosynthesis is functioning well, it results in higher levels of carbohydrates, fats, and proteins. Carbohydrates, fats, and proteins are the components that make up soybean seeds, and the higher their levels, the greater the number of seeds produced, thus affecting the weight of seeds produced (Anisa et al., 2022).

The research results show that the PGR treatment with Atonik at a concentration of 1 ml/l (Z2) resulted in a weight of seeds each plant of 9.34 g, which is not significantly different from the concentration of 0 ml/l of Atonik (Z0), the concentration of 1.5 ml/l of Atonik (Z3), but significantly different from the concentration of 0.5 ml/l of Atonik (Z1). The concentration of 1 ml/l of Atonik (Z2) produced 2.86% more weight compared to the concentration of 0 ml/l of Atonik (Z0), 22.89% more weight compared to the concentration of 0.5 ml/l of Atonik (Z1), and 5.66% more weight compared to the concentration of 1.5 ml/l of Atonik (Z3).

Table 2 also shows that the difference in soybean lines significantly affects the weight of seeds each plant. This is likely due to the variations and results of each genotype caused by their different adaptation abilities, even when grown in the same area. The genotypes respond differently to environmental conditions, such as water availability (Josipović et al., 2011). The GH 8 line (G1) produced the highest number of seeds each plant, which is 62.16 g, resulting in the heaviest weight of seeds each plant, which is 9.61 g, not significantly different from the Dega 1 (G4) at 9.90 g.

The research results show that the treatment with the Dega 1 soybean (G4) resulted in the highest weight of seeds each plant, which is 9.90 g, significantly different from the GH 63 (G2), the GH 73 P (G3), but not significantly different from the GH 8 (G1). The Dega 1 produced 3.02% more weight compared to the GH 8 (G1), 19.71% more weight compared to the GH 63 (G2), and 33.13% more weight compared to the Dega 1 (GH 4).

3.5 Weight of 100 Seeds

Table 2 shows that the Atonik PGR concentration significantly affects the weight of 100 seeds. This is likely related to the individual seed weight produced each plant. At a concentration of 1 ml/l of Atonik (Z2), the weight of seeds each plant is 9.34 g, which is not significantly different from the concentration of 1.5 ml/l of Atonik (Z3), resulting in a weight of 8.91 g each plant. However, the concentration of 1.5 ml/l of Atonik (Z3) produced a weight of 100 seeds amounting to 23.21 g, indicating that individual seed weight at this concentration is heavier than that of 1 ml/l of Atonik (Z2). The use of plant growth regulators containing auxin can affect the photosynthesis process in plants.

The research results demonstrate that the PGR treatment with Atonik at a concentration of 1.5 ml/l (Z3) resulted in a weight of 100 seeds of 23.21 g, significantly different from the concentration of 0 ml/l (Z0), the concentration of 0.5 ml/l of Atonik (Z1), and the concentration of 1 ml/l of Atonik (Z2). The concentration of 1.5 ml/l produced 34.16% more weight compared to GH 8 (G1), 23.26% more weight compared to GH 63 (G2), and 21.03% more weight compared to GH 73 P (G3).

Table 2 also indicates that the different soybean lines significantly affect the weight of 100 seeds. This is related to the weight of seeds each plant each strain produces. The GH 8 (G1) produced a weight of 9.61 g each plant, which is not significantly different from the Dega 1 (G4) producing 9.90 g each plant. The GH 8 (G1) had the highest weight of 100 seeds, which is 20.49 g. The difference in seed weight is affected by genetic and environmental factors, where the instability in seed size of each genotype is simultaneously affected by genetic and environmental factors (Liu et al., 2004).

The research results show that the GH 8 (G1) produced the highest weight of 100 seeds, which is 20.49 g, not significantly different from the GH 63 (G2), the GH 73 P (G3), but significantly different from the Dega 1 (G4). The GH 8 produced 4.27% more weight compared to the GH 63 (G2), 2.86% more weight compared to the GH 73 P (G3), and 9.89% more weight compared to the Dega 1 (G4).

IV. CONCLUSION

Based on the results of the discussion above, the following conclusions can be drawn: A concentration of 1 ml/l of the plant growth regulator Atonik in water resulted in the highest number of productive branches each plant, which is 5.25 branches. It also led to the highest number of seeds each plant, 55.14 seeds, and the heaviest seed weight each plant, 9.34 g. On the other hand, a concentration of 1.5 ml/l of Atonik in water resulted in the heaviest weight of 100 seeds each plant, which is 23.21 g. Among the soybean lines tested, GH 63 produced the highest number of leaves each plant, which is 92.77 leaves, and the highest number of productive branches each plant, which is 5.25 branches. GH 8 produced the highest number of seeds each plant, 62.16 grains, and the heaviest weight of 100 seeds each plant, 20.49 g. Lastly, the soybean Dega 1 resulted in the heaviest seed weight each plant, which is 9.90 g.

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