LAPORAN TAHUN TERAKHIR
PENELITIAN TERAPAN UNGGULAN PERGURUAN TINGGI
(PTUPT)

Pemanfaatan Tanaman Tembakau (*Nicotiana tabacum*) Toleran Genangan Pada Cekaman Genangan Secara Periodik Sebagai Bahan Bioinsektisida Yang Ramah Lingkungan

TAHUN KE – 2 DARI RENCANA 2 TAHUN

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SESUAI DENGAN PERJANJIAN PENDANAAN PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT
NOMOR: 122/SP2H/PTNBH/DRPM/2018

UNIVERSITAS AIRLANGGA
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HALAMAN PENGESAHAN

Judul	: Pemanfaatan Tanaman Tembakau (Nicotiana tabacum) Toleran Genangan Pada Cekaman Genangan Secara Periodik Sebagai Bahan Bioinsektisida Yang Ramah Lingkungan
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RINGKASAN

Dalam dunia pertanian di negara kita saat ini pemanfaatan pestisida kimia sebagai agen pembunuh hama penyerang tanaman semakin meningkat dosis dan frekuensi pemakaiannya. Pestisida kimia digunakan karena pestisida tersebut mudah didapat dan sangat cepat serta ampuh memberantas hama. Akan tetapi penggunaan pestisida kimia juga dapat menimbulkan dampak negatif bagi tanaman maupun bagi lingkungan sekitar tanaman serta berdampak negatif juga bagi manusia. Berdasarkan dampak yang ditimbulkan dari penggunaan pestisida kimia tersebut maka, perlu dilakukan penelitian untuk mendapatkan bioinsektisida dengan menggunakan bahan-bahan yang tersedia di Indonesia yang ramah lingkungan. Salah satu bahan yang dapat digunakan sebagai bioinsektisida adalah tembakau (*Nicotiana tabacum*). Untuk menjaga keberlangsungan dan ketersedian tembakau sebagai bahan baku bioinsektisida maka kualitas dan budidaya tembakau merupakan parameter penting yang harus diperhatikan.

Salah satu kendala dalam kegiatan budidaya tembakau adalah hujan yang turun tidak menentu. Selain itu, kendala lain adalah terjadinya intensitas hujan yang tinggi. Pada daerah tropis dengan curah hujan yang tinggi seringkali terjadi genangan baik yang bersifat temporer maupun yang berlangsung dalam periode yang relatif lama. Sehingga tanaman tembakau toleran genangan perlu dikaji untuk mendapatkan kualitas tanaman yang optimal sebagai stok untuk bahan baku bioinsektisida. Dalam kondisi tercekam seperti genangan tanaman tembakau memiliki senyawa bioaktif yang meningkat seperti nikotin. Sehingga penelitian ini bertujuan mengetahui aplikasi bioinsektisida tanaman tembaku toleran pada cekaman genangan secara periodik pada daya hambat maupun daya bunuh insekta penggangu tanaman.

Rancangan yang digunakan dalam penelitian ini adalah rancangan acak lengkap dengan 3 kali ulangan. Konsentrasi ekstrak yang digunakan adalah 9 konsentrasi ekstrak daun ketapang (*Terminalia catappa*) dan 1 kontrol, kemudian dari 10 konsentrasi yang akan diujikan selama 3 hari dicari nilai ambang batas bawah LC10 dan ambang batas atas LC90 serta LC_{50}, pengamatan pola mortalitas, persentase pembentukan pupa serta uji kandungan antifeedant.

Hasil penelitian adalah (1) Ekstrak daun Tembakau berpotensi sebagai insektisida nabati.; (2) Ekstrak daun tembakau berpengaruh terhadap mortalitas *Spodoptera litura* instar 3 dan didapatkan LC50 didapatkan pada konsentrasi 47% (47 gr/ 100 ml) dalam kurun waktu 48 jam pengamatan. Target luaran adalah Jurnal Internasional dan Seminar Internasional dan produk biopestisida ekstrak daun tembakau.

Kata Kunci: Tembakau (*Nicotiana tabacum*), Toleran Genangan, Genangan Secara periodik, Bioinsektisida

PRAKATA

Puji syukur ke hadirat Allah SWT berkat limpahan rahmat dan hidayah-NYA sehingga penelitian pada Tahun pertama yang berjudul **"Pemanfaatan Tanaman Tembakau (*Nicotiana tabacum*) Toleran Genangan Pada Cekaman Genangan Secara Periodik Sebagai Bahan Bioinsektisida Yang Ramah Lingkungan "** dapat terselesaikan.

Peneltian ini merupakan Hibah Penelitian Desentralisasi Skema Penelitian Terapan Unggulan Perguruan Tinggi Tahun Anggaran 2018 dengan Nomor: SK: 01/E/KPT/2018 dengan No Kontrak: 200/UN3.14/LT/2018

Banyak pihak telah membantu penelitian ini, untuk itu pada kesempatan ini penulis mengucapkan terima kasih atas bantuan yang telah diberikan, kepada:

1. Direktur Penelitian Dan Pengabdian Kepada Masyarakat
2. Ketua Lembaga Penelitian Dan Inovasi UNAIR
3. Dekan FST Universitas Airlangga

Peneliti telah berusaha semaksimal mungkin agar hasil penelitian dapat menghasilkan sesuatu yang bermanfaat. Namun tak ada gading yang tak retak dengan tangan terbuka penulis berharap adanya kritik dan saran untuk perbaikan laporan ataupun penelitian selanjutnya.

Surabaya, 12 Nopember 2018

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BAB 1. PENDAHULUAN

1.1 Latar Belakang

Peningkatan pembangunan pertanian di Indonesia, menyebabkan kebutuhan akan pestisida semakin meningkat, baik jumlah maupun jenisnya. Sejak tahun permulaan pelaksanaan program intensifikasi pangan, masalah hama diusahakan ditanggulangi dengan berbagai jenis formulasi pestisida. Orientasi pemerintah pada waktu itu tertumpu pada peningkatan hasil sebanyak-banyaknya, tanpa memperhatikan dampak negatif terhadap lingkungan. Selain itu dengan dicanangkannya program intensifikasi pangan melalui program nasional BIMAS, pestisida telah dimasukkan sebagai paket teknologi yang wajib digunakan oleh petani. Kebijakan tersebut, akhirnya merangsang petani menggunakan pestisida. Jenis pestisida yang dianjurkan digunakan pada waktu itu umumnya adalah pestisida yang berdaya bunuh berspektrum luas, yaitu mampu membunuh sebagian besar organisma yang dikenainya, termasuk organisma berguna seperti musuh alami hama dan organisma bukan target lainnya yang hidup berdampingan dengan organisma pengganggu tanaman. Program penyuluhan pertanianpun merekomendasikan aplikasi pestisida secara terjadwal dengan sistem kalender, tanpa memperhatikan ada atau tidak ada hama yang menyerang tanaman di lapangan. Kondisi tersebut menyebabkan frekuensi penyemprotan menjadi lebih intensif, dan biasa dilakukan setiap minggu sepanjang musim tanam (Girsang, 2009).

Beberapa kerugian yang muncul akibat pengendalian organisma pengganggu tanaman yang mengandalkan pestisida, antara lain menimbulkan kekebalan (resistensi) hama, mendorong terjadinya resurgensi, terbunuhnya musuh alami dan jasad non target, serta dapat menyebabkan terjadinya ledakan populasi hama sekunder. Menyikapi permasalahan tersebut perlu dicari cara pengendalian alternatif yang lebih aman, dengan harga terjangkau tetapi memiliki efektivitas yang relatif tidak berbeda dengan pengendalian menggunakan pestisida sintetis. Salah satu teknik yang dapat diterapkan adalah pemanfaatan bioinsektisida berbahan aktif yang berasal dari tanaman (Javed et al. 2006).

Indonesia memiliki flora yang sangat beragam, mengandung cukup banyak jenis tumbuh-tumbuhan yang merupakan sumber bahan insektisida yang dapat dimanfaatkan untuk pengendalian hama. Salah satu tanaman yang berpotensi sebagai bioinsektisida tersebut adalah tembakau (*Nicotiana tabacum*). Potensi tersebut disebabkan adanya kandungan nikotin yang terdapat pada tanaman tembakau. Peningkatan kebutuhan nikotin tembakau perlu diikuti oleh peningkatan hasil dan kualitas tanaman pada budidaya tembakau.

Di sisi lain, tembakau merupakan tanaman yang sangat sensitif terhadap cara budidaya, lokasi tanam, musim/cuaca, dan cara pengolahan. Adanya perubahan iklim yang tidak menentu menuntut terciptanya tembakau yang toleran terhadap cekaman abiotik, seperti genangan atau banjir. Perubahan iklim dan cuaca pada saat ini sungguh berubah drastis, memanjangnya musim penghujan sangat meresahkan petani tembakau yang ada di Indonesia. Tembakau merupakan tanaman yang sangat peka terhadap kelebihan air, karena dapat menyebabkan kelayuan tiba-tiba (lengger) sebagai akibat kekurangan oksigen dan tanaman yang membusuk. Membusuknya tanaman akan membuat populasi tanaman tembakau semakin berkurang sehingga pada akhirnya produksi hasil panen tidak akan maksimal yang selanjutnya akan berdampak pada kandungan nikotin sebagai bahan baku insektisida (Nur & Apriana, 2013).

Genangan atau banjir termasuk cekaman lingkungan abiotik yang dapat menghambat pertumbuhan tanaman (Taiz & Zeiger, 2010). Besarnya penurunan laju pertumbuhan tanaman yang tergenang ditentukan oleh tingkat genangan. Cekaman genangan terbagi menjadi beberapa tingkat, yaitu: cekaman genangan yang hanya terjadi pada sistem perakaran (*waterlogging*), cekaman genangan yang merendam semua akar dan sebagian batang tanaman (*partial submergence*) dan seluruh bagian tanaman terendam air (*complete submergence*) (Shimamura *et al.*, 2007 dan Striker & Mworia, 2012). Cekaman genangan memiliki dampak terhadap proses fisiologis tanaman yang pada akhirnya juga akan mempengaruhi pada produktivitas sekaligus nikotin tanaman (McLuckie *et al.*, 2007).

Respon tumbuhan terhadap genangan bervariasi sesuai dengan ketahanannya terhadap genangan. Menurut Taiz & Zeiger (2010) pengelompokan tumbuhan berdasarkan ketahanannya terhadap genangan terbagi menjadi tumbuhan yang sensitif terhadap genangan dan tumbuhan yang toleran terhadap genangan. Tumbuhan sensitif akan mengalami kerusakan apabila mengalami anoksia selama 24 jam sedangkan tumbuhan toleran dapat bertahan pada kondisi anoksia untuk sementara, tetapi tidak dapat bertahan pada kondisi anoksia yang lebih lama. Sarkar *et al.* (2006) melaporkan bahwa toleransi genangan merupakan adaptasi tanaman dalam merespon proses anaerob yang memampukan sel untuk mengatur atau memelihara keutuhannya sehingga tanaman mampu bertahan hidup dalam kondisi sedikit oksigen (hipoksia) tanpa kerusakan yang berarti. Tanaman mampu hidup dan tumbuh pada kondisi tanah tergenang diantaranya melalui adaptasi morfologi, fisiologi dan molekuler (Pourabdal, 2008 *dalam* Susilawati dkk., 2012).

Pada penelitian yang pertama sudah dilakukan pengamatan faktor morfologi dan fisiologi terhadap tanaman tembakau pada cekaman periodic sebagai bahan bioinsektisida. Berdasarkan penelitian yang pertama dapat diketahui bahwa respon morfologi dan fisiologi ini berpengaruh pada tanaman tembakau sehingga diduga juga mempunyai pengaruh terhadap kandungan nikotin tanaman tebakau, yang pada akhirnya akan berpengaruh pada kemampuan bioinsektisidanya.

Selain kedua respon tersebut tanaman tembakau yang tercekam genangan juga akan berpengaruh pada molekulernya yaitu terkait dengan gen yang bertanggungjawab ketika tanaman berada dalam kondisi cekaman gengan. Beberapa gen yang terlibat dalam mekanisme resistensi/toleransi merupakan gen-gen yang umum diekspresikan tanaman dalam kondisi stres, namun beberapa gen secara spesifik diekspresikan pada kondisi cekaman lingkungan tertentu. Ekspresi suatu gen ditandai oleh terbentuknya massenger RNA (mRNA). Gen-gen yang berperan dalam mekanisme resistensi/toleransi termasuk didalam kelompok gen-gen yang ekspresinya teregulasi/terinduksi. Gen-gen tersebut pada umumnya hanya diekspresikan/diekspresikan secara berlebih (overexpression) bila tanaman (dalam pertumbuhan/perkembangannya) mengalami kondisi cekaman lingkungan tertentu seperti cekaman genangan. Penanaman tanaman pada kondisi cekaman tertentu akan menginduksi transkripsi mRNA dari gen-gen yang diduga berperan dalam mekanisme resistensi/toleransi tanaman terhadap cekaman tersebut. Salah satu gen yang terlibat adalah enzim XET (xyloglucan endo translycosylase) yang merupakan enzim peluruh dinding putatif selama masa perkecambahan, perkembangan jaringan serta pelunakan buah sehingga membentuk jaringan aerenkim (Saab and Sachs 1996).

Sehingga pada penelitian selanjutnya akan dilakukan pengamatan secara molekuler terhadap gen tanaman tembakau tercekam genangan sebagai bahan baku bionsektisida dan aplikasi bioinsektisida pada tanaman yang diinfeksi oleh insektisida.

Berdasarkan uraian di atas menunjukkan bahwa semua perubahan yang terjadi baik perubahan fisiologi maupun morfologi maupun molekuler pada tanaman tembakau sangat mempengaruhi kemampuan tanaman untuk bertahan hidup pada kondisi tergenang. Tanaman tembakau yang toleran pada kondisi tergenang mampu memberikan kontribusi yang besar terhadap ketersediaan nikotin sebagai bahan baku bioinsektisida.

1.2 Perumusan Masalah

Untuk mendukung pemanfaatan tanaman tembakau sebagai bahan bioinsektisida diperlukan pengetahuan tentang perubahan sifat-sifat molekuler tanaman temabakau pada kondisi tergenang. Hal ini disebabkan tembakau adalah tanaman yang sangat sensitif terhadap musim / cuaca. Berdasarkan kenyataan yang telah disampaikan diatas, dapat dirumuskan masalah sebagai berikut:

1. Bagaimanakah karakter molekuler tanaman tembakau yang toleran pada cekaman genangan secara periodik sebagai kandidat bahan baku bioinsektisida?
2. Bagaimanakah pengaruh bioinsektisida tanaman tembakau yang toleran pada cekaman genangan secara periodik pada tanaman yang terserang oleh insekta?

1.3 Pembatasan Permasalahan

Batasan masalah dalam penelitian ini adalah:

1. Varietas Tanaman Tembakau yang digunakan adalah jepon Palakean, Srumpung dan Sumporis
2. Cekaman Genangan Secara Periodik Pada Tingkat *waterlogging* sampai *Flodding Partial subemergence* dengan durasi 14 hari
3. Parameter terukur meliputi Karakter molekuler tanaman tembakau yang tercekam genangan srta karakter morfologi dan fisiologi tanaman uji yang diberi aplikasi bioinsektisida tanaman tembakau yang tercekam genangan.

1.4 Urgensi Penelitian

Dalam dunia pertanian di negara kita saat ini pemanfaatan pestisida kimia sebagai agen pembunuh hama penyerang tanaman semakin meningkat dosis dan frekuensi pemakaiannya. Pestisida kimia digunakan karena pestisida tersebut mudah didapat dan sangat cepat serta ampuh memberantas hama. Akan tetapi penggunaan pestisida kimia juga dapat menimbulkan dampak negatif bagi tanaman maupun bagi lingkungan sekitar tanaman serta berdampak negatif juga bagi manusia. Berdasarkan dampak yang ditimbulkan dari penggunaan pestisida kimia tersebut maka, perlu dilakukan penelitian untuk mendapatkan bioinsektisida dengan menggunakan bahan-bahan yang tersedia di Indonesia yang ramah lingkungan. Salah satu bahan yang dapat digunakan sebagai bioinsektisida adalah tembakau (*Nicotiana tabacum*). Hal ini dikarenakan tembakau mengandung senyawa alkaloid utama berupa nikotin (Simpson

& Ogorzaly, 2001). Dalam bentuk kering, tembakau mengandung 1% sampai 3% nikotin. Nikotin biasa digunakan untuk bioinsektisida (Stern dkk., 2003). Untuk menjaga keberlangsungan dan ketersedian tembakau sebagai bahan baku bioinsektisida maka kualitas dan budidaya tembakau merupakan parameter penting yang harus diperhatikan.

Salah satu kendala dalam kegiatan budidaya tembakau adalah hujan yang turun tidak menentu. Selain itu, kendala lain adalah terjadinya intensitas hujan yang tinggi. Pada daerah tropis dengan curah hujan yang tinggi seringkali terjadi genangan baik yang bersifat temporer maupun yang berlangsung dalam periode yang relatif lama (Susilawati dkk., 2012). Genangan merupakan penyebab cekaman hipoksia atau anoksia pada tanaman (Smith dkk., 2010). Genangan yang terjadi menyebabkan kondisi perakaran tanaman menjadi anaerob (Hodson & Bryant, 2012). Genangan merupakan cekaman lingkungan abiotik yang dapat menghambat pertumbuhan tanaman (Taiz & Zeiger, 2010). Besarnya penurunan laju pertumbuhan tanaman yang tergenang ditentukan oleh tingkat genangan. Cekaman genangan terbagi menjadi beberapa tingkat, yaitu: cekaman genangan yang hanya terjadi pada sistem perakaran, cekaman genangan yang merendam semua akar dan sebagian batang tanaman, dan cekaman genangan yang merendam seluruh bagian tanaman (Striker, 2012). Kondisi tersebut akan mempengaruhi kualitas dan pertumbuhan tanaman tembakau, sehingga tanaman perlu melakukan adaptasi.

Adaptasi tanaman agar mampu hidup dan tumbuh pada kondisi tergenang diantaranya melalui adaptasi morfologi dan fisiologi (Pourabdal *dalam* Susilawati *et al.*, 2012). Oleh karena itu karakter morfologi dan fisiologi tanaman tembakau pada kondisi tergenang merupakan hal yang penting dilakukan, untuk mendapatkan tanaman tembakau yang toleran terhadap genangan. Hal tersebut dilakukan mengingat kondisi iklim dengan frekuensi curah hujan yang meningkat akan berpengaruh terhadap pertumbuhan tanaman, sehingga keberlanjutan pemanfaatan tembakau sebagai bahan bioinsektisida tetap terjaga.

BAB 2. TINJAUAN PUSTAKA

2.1 RENSTRA PERGURUAN TINGGI

Lembaga Penelitian dan Inovasi (LPI) Universitas Airlangga dibentuk berdasarkan Keputusan Rektor nomor 1280/UN3/KR/2015, tanggal 20 Agustus 2015. Sehubungan dengan penetapan Universitas Airlangga sebagai Perguruan Tinggi
Negeri Berbadan Hukum (PTN BH), maka tugas utamanya adalah mengembangkan penelitian dan mengarahkan hasil produk penelitian yang inovatif agar dapat dimanfaatkan masyarakat.

Dalam rangka mencapai academic milestones yang telah ditentukan maka kegiatan penelitian dan inovasi didasarkan pada pengembangan budaya ilmiah di lingkungan Universitas Airlangga yang berbasis pada health sciences, biosciences dan social sciences yang ketiganya bersifat interdependent. Oleh karena itu
disusunlah Rencana Induk Penelitian (RIP) Universitas Airlangga yang di dalamnya terdapat 17 tema riset unggulan universitas dan roadmap penelitiannya. RIP Universitas Airlangga dibuat agar dapat menjadi arah kebijakan dan pengambilan keputusan dalam pengelolaan penelitian institusi dalam jangka waktu 5 tahun mulai dari tahun 2016 hingga 2020. Harapan yang ingin dicapai adalah agar dalam kurun waktu tertentu akan dicapai hasil yang jelas dan terencana untuk 3 tujuan penelitian yaitu pengembangan keilmuan, dukungan peningkatan kualitas institusi dan penyelesaian permasalahan yang terjadi di masyarakat.

Penyusunan road mapp enelitian ini juga bertujuan untuk mengembangkan budaya ilmiah pada penelitian yang berorientasi pada pencapaian produk unggulan, baik bioexcellent-product maupun model excellent, sehingga membawa manfaat nyata, baik untuk kepentingan institusi maupun masyarakat dengan keluaran berupa teknologi, produk maupun market yang berlandaskan pada hasil riset.

Penelitian juga diarahkan pada model kerjasama nasional dan internasional baik secara institusional yang menyangkut finansial maupun tidak. Kerjasama penelitian didasarkan pada prinsip mutualisme untuk menghasilkan produk unggulan dan internasionalisasi bidang penelitian yang dapat diimplementasikan dalam program pengabdian kepada masyarakat dalam rangka mencapai academic milsetones.

2.2 ROAD MAP PENELITIAN PERGURUAN TINGGI

Tema Riset Dan Unggulan Universitas Airlangga

No	Bidang / Fakulatas	Tema Riset Unggulan
1	Pertanian	1. Pemberdayaan Masyarakat Pesisir dan feriferal 2. Ketahanan Pangan
2	Kesehatan dan Obat	3. Pengembangan obat dan Bahan Alam 4. Kanker dan Autoimun 5. Penanggulangan Penyakit Tropis 6. Pengembangan Stem Cel
3	Sosial Ekonomi dan Hukum	7. Sistem pengelolaan layanan kesehatan penduduk miskin 8. Pengembangan Regulasi dan model kebijakan 9. Pemilu dan demokrasi
4	Matematika dan Ilmu Pengetahuan Alam	10. Pengembangan material maju 11. Produksi Tanaman Transgenik 12. Produk Hasil Fermentasi mikroorganisme 13. Bioremediasi lingkungan dan pengelolaan limbah 14. Pemodelan di bidang life science, ekonomi dan industri berbasis ict
5	Psikologi dan Budaya	15. Integrasi dan Harmonisasi Nasional 16. Seni dan budaya untuk menunjang industri kreatif 17. Pembangunan Manusia dan Daya saing

Kompetensi Keahlian	Isu strategis	Konsep Penelitian	Pemecahan Masalah	Topik Penelitian Fakultas
Natural Science Farmasi Ekonomi Kedokteran Hewan	Insektisida saat ini yang digunakan menggunakan berbagai bahan kimia yang merusak lingkungan	Mengubah Pola Penggunaan insektisida kimia dengan bioinsektisida ramah lingkungan	Perlu dilakukan berbagai penelitian untuk mendapatkan bioinsektisida dengan menggunakan bahan-bahan yang tersedia di Indonesia yang ramah lingkungan	Eksplorasi bioinsektisida ramah lingkungan dengan menggunakan bahan yang berasal berbagai tumbuhan yang terdapat di Indonesia
				Ekonomi Pertanian

2.3 Road Map Penelitian Yang Mendukung

Tahap	Fokus	Keluaran	Pelaksana Dan Tahun Pelaksanaan
Eksporasi Tanaman Berpotensi Insektisida	Tanaman Budidaya Yang Bukan Tanaman Pangan	Tanaman Berkandidat Bioinktisida	2003 – 2005 oleh Dosen Dan Mahasiswa
Cekaman Biotik Dan Abiotik Dalam Meningkatkan Bahan aktif Untuk Bioinsktisida	Cekaman kekeringan, cekaman genangan, cekaman salinita dan cekaman biotik	Karakter morfologi, fisiologi dan molekuler tanaman yang dikandidatkan sebagai bioinsektisida	2006 – 2009 oleh Dosen Dan Mahasiswa
Aplikasi Bioinsektisida pada Tanaman Budidaya	Tanman budidaya yang terserang insektisida	Produk berupa insektisida	2016 – 2020 oleh Dosen Dan Mahasiswa

No	Judul Penelitian	HASIL PENELITIAN
	Studi Potensi Bioherbisida Ekstrak Daun Ketapang (Terminalia catappa) terhadap Gulma Rumput Teki (Cyperus rotundus) (Tahun 2012)	Hasil dari penelitian ini adalah ekstrak daun ketapang (*T. catappa*) dapat digunakan sebagai salah satu alternatif untuk menghambat pertumbuhan tinggi gulma rumput teki (*C. rotundus*) serta konsentrasi ekstrak daun ketapang (*T. catappa*) yang dapat digunakan sebagai salah satu alternatif untuk menghambat pertumbuhan tinggi gulma rumput teki (*C. rotundus*) adalah konsentrasi 50% .
2	**Pengaruh Ekstrak Daun Bintaro (Cerbera odollam) terhadap Perkembangan Ulat Grayak (Spodoptera litura F.) (Tahun 2013):**	Hasil penelitian menunjukkan ekstrak daun *Cerbera odollam* konsentrasi 2% di hari kedelapan pengamatan dapat menurunkan berat tubuh *S. litura* F.. Konsentrasi 2% dari ekstrak daun *Cerbera odollam* juga menghambat proses ekdisis pada instar 2 sampai instar 3 dan dapat menghambat pembentukan pupa. .
3	**Short Communication:**	The Probit analysis resulted that 50% of lethal concentration

	Larvicidal and antifeedant activities of Kalanchoe daigremontiana against Plutella xylostella larvae (Tahun 2016)	(LC50) was found at 72 hours exposure of 0,5% polar extract. Effective concentra tion for antifeedant at 1.25%, while antifeedant capacity percentage was in level of 84.85%.
4	**EXPLORATION OF POTENTIAL PLANTS AS A BIO-INSECTICIDE AT ITS SURABAYA CAMPUS (Tahun 2015)**	Ten plants species with bio-insecticide potential in this study were *Ageratum conyzoides* L., *Crynum asiaticum* L., *Calotropis gigantea* R., *Eugenia cumini* Merr., *Eichornia crassipes*, *Crescentia cujete* L., *Nothopanax scutellarium* Merr., *Morinda citrifolia* L., *Azadirachta indica*, and *Lantana camara* L.
5	**Pemanfaatan Tanaman Tembakau (Nicotiana tabacum) Toleran Genangan Pada Cekaman Genangan Secara Periodik Sebagai Bahan Bioinsektisida Yang Ramah Lingkungan (Tahun 2017)**	Berdasarkan hasil penelitian sementaraa didapatkan hasil bahwa pada karakter morfologi yang meliputi (tinggi tanaman, jumlah daun, luas daun, panjang akar, dan jumlah akar adventif). Berdasarkan analisis one Way untuk karakter morfologi memiliki kecenderungan yang sama bahwa cekaman genangan secara periodik yang diberikan memberikan pengaruh yang cenderung menurunkan pertumbuhan tanaman tembakau untuk beberapa varietas tertentu. Namun untuk jumlah akar adventif dengan cekaman yang semakin meningkat dari tingkat waterlogging yang kemudian ditingkatkan secaraa flooding sub mergence mengalami peningkatan sebagai bentuk adaptasi tumbuhan yang tercekam genangan. Sedangkan pada karakter fiologi yang meliputi (kandungan klorofil daun, kadar etilen akar), dengan adanya cekaman genangan secara periodik akan menurunkan kadar klorofil dan meningkatkan kadar etilen *pada tanaman tembakau.*

2.4 Tanaman Tembakau (*Nicotiana tabacum* L.)

Di Indonesia, terdapat daerah-daerah tertentu yang memiliki kualitas tembakau yang baik dan komersial. Beberapa jenis tembakau di Indonesia adalah tembakau Deli yang ditanam di Medan dan sekitarnya, tembakau Vorstenland yang ditanam antara Solo-Yogyakarta, tembakau Besuki yang ada di daerah Jember, tembakau Virginia yang ditanam di Jawa Timur, Lombok dan di Sulawesi Selatan, tembakau Ampenan di Pulau Lombok, tembakau Cabenge di Sulawesi Selatan, tembakau Payakumbuh di Sumatera Barat, tembakau Mole di Garut, Jawa Barat, tembakau Kedu di Jawa Tengah, tembakau Kasturi di Jember, dan tembakau Madura di Madura (Santoso, 2001).

Tembakau yang diperuntukkan untuk konsumsi dalam negeri merupakan tembakau asli atau tembakau rakyat, seperti tembakau Temanggung dan Kendal di Jawa Tengah, tembakau Madura di Jawa Timur dan tembakau jenis Virginia yang terdapat di Jawa Tengah, Bojonegoro (Jawa Timur), Bali dan Lombok (Dirjen Perkebunan RI *dalam* Rochadi, 2005).

Secara umum tembakau di Indonesia dapat dibedakan menurut musim tanamnya yang terbagi menjadi dua jenis yaitu:

a. Tembakau *Voor-Oogst*

Tembakau *Voor-Oogst* biasanya dinamakan tembakau musim kemarau. Jenis tembakau ini ditanam pada akhir musim penghujan dan dipanen pada waktu musim kemarau (Peraturan Menteri Pertanian, 2012). Tanaman tembakau jenis *Voor-Oogst* ini adalah jenis tembakau Virginia, tembakau rakyat (Jawa), dan tembakau Lumajang (Pemerintah Kabupaten Lamongan, 2008 *dalam* Rachmawati dkk., 2013).

b. Tembakau *Na-Oogst*

Tembakau *Na-Oogst* yaitu jenis tembakau yang ditanam akhir musim kemarau, kemudian dipanen atau dipetik pada musim penghujan (Peraturan Menteri Pertanian, 2012). Tanaman tembakau jenis ini adalah jenis tembakau Besuki (Pemerintah Kabupaten Lamongan, 2008 *dalam* Rachmawati dkk., 2013).

Jawa Timur memiliki lokasi pengembangan yang potensial seperti wilayah kabupaten Bojonegoro, Lamongan, Bondowoso, Jember, Blitar dan lain-lain. Varietas-varietas yang sering ditanam petani di Jawa Timur adalah Grompol Jatim 1, Kasturi 1, Kasturi 2, Coker 176, Cangkring 45, Kemloko 1, Kemloko 2, Kemloko 3, DB 101, TN 90, Paiton 1, Paiton 2, Maesan 1, Sindoro 1, Prancak N-1, Prancak N-2, Prancak 95, Bligon 1, Bojonegoro 1, Jepon Pelakean, Jinten, Manilo dan Marakot (Purdyaningsih, 2013).

Pada tahun 2013, penanaman tembakau jawa di Jawa Timur tersebar di 26 kabupaten. Dari 26 kabupaten yang memproduksi tembakau jawa tersebut, terdapat 6 kabupaten terbesar dengan total kontribusi mencapai 75,40% dari total produksi tembakau jawa di Jawa Timur yaitu Bondowoso, Jombang, Lamongan, Situbondo, Nganjuk dan Tulungagung (Ekanantari, 2014). Susunan taksonomi tanaman tembakau / *Nicotiana tabacum* L. menurut Simpson (2010) sebagai berikut:

Regnum	: Plantae
Divisio	: Magnoliophyta
Classis	: Magnoliopsida
Ordo	: Solanales
Familia	: Solanaceae
Genus	: Nicotiana
Species	: *Nicotiana tabacum* L.

2.5 Biopestisida Nabati

Pestisida nabati adalah pestisida yang bahan aktifnya berasal dari tumbuhan atau bagian tumbuhan seperti akar, daun, batang atau buah. Bahan-bahan ini diolah menjadi berbagai bentuk, antara lain bahan mentah berbentuk tepung, ekstrak atau resin yang merupakan hasil pengambilan cairan metabolit sekunder dari bagian tumbuhan atau bagian tumbuhan dibakar untuk diambil abunya dan digunakan sebagai pestisida (Thamrin *dkk*, 2005).

Pestisida dari bahan nabati sebenarnya bukan hal yang baru tetapi sudah lama digunakan, bahkan sama tuanya dengan pertanian itu sendiri. Sejak pertanian masih dilakukan secara tradisional, petani di seluruh belahan dunia telah terbiasa memakai bahan yang tersedia di alam untuk mengendalikan organisme pengganggu tanaman. Pada tahun 40-an sebagian petani di Indonesia sudah menggunakan bahan nabati sebagai pestisida, diantaranya menggunakan daun sirsak untuk mengendalikan hama belalang dan penggerek batang padi. Sedangkan petani di India, menggunakan biji mimba sebagai insektisida untuk mengendalikan hama serangga. Namun setelah ditemukannya pestisida sintetik pada awal abad ke-20, pestisida dari bahan tumbuhan atau bahan alami lainnya tidak digunakan lagi. (Thamrin *dkk*, 2005).

Pestisida nabati dapat dibuat dengan menggunakan teknologi yang sederhana yang dikerjakan oleh kelompok tani atau petani perorangan. Pestisida nabati yang dibuat secara sederhana hasilnya dapat berupa larutan hasil perasan, rendaman, ekstrak dan rebusan dari bagian tanaman berupa akar, umbi, batang, daun, buah dan biji. Apabila dibandingkan dengan pestisida kimia, penggunaan pestisida nabati relative aman dan murah. Beberapa tanaman yang dapat digunakan sebagai pestisida nabati, yang dapat dibuat melalui teknologi yang sederhana adalah Mimba, biji srikaya, sirih dan lain-lain (Rachmawaty *dan* Korlina, 2009).

Sampai saat ini telah terinventarisasi sebanyak 2.400 jenis tumbuhan yang terdiri dari 235 famili berpotensi sebagai bahan pestisida nabati. Famili tumbuhan yang dianggap merupakan sumber potensial insektisida nabati adalah Meliaceae, Annonaceae, Asteraceae, Piperaceae, Rutaceae. Namun hal ini tidak menutup kemungkinan untuk ditemukannya famili tumbuhan yang baru untuk dijadikan sebagai insektisida nabati (Rachmawaty *dan* Korlina, 2009).

Pada umumnya pestisida sintetik dapat membunuh langsung organisme sasaran dengan cepat. Hal ini berbeda dengan pestisida nabati, sebagai contoh insektisida nabati yang umumnya tidak dapat mematikan langsung serangga, biasanya berfungsi seperti berikut: (1)Refelen, yaitu menolak kehadiran serangga terutama disebabkan baunya yang menyengat; (2) Antifidan, menyebabkan serangga tidak menyukai tanaman, misalnya disebabkan rasa yang pahit; (3) Mencegah serangga meletakkan telur dan menghentikan proses penetasan telur; (4) Racun syaraf; (5) Mengacaukan sistem hormon di dalam tubuh serangga; (6) Attraktan, sebagai pemikat kehadiran serangga yang dapat digunakan sebagai perangkap.

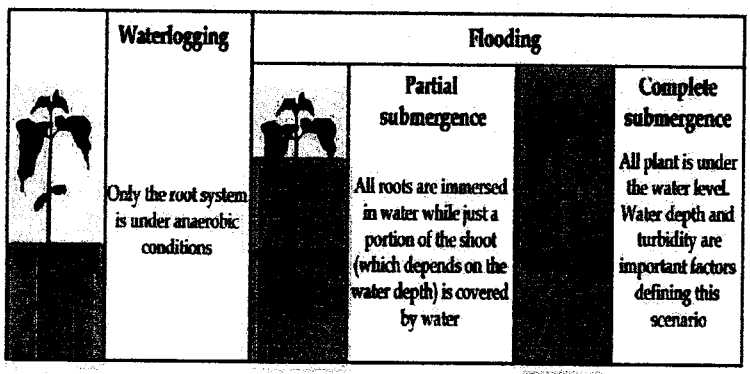
Pestisida nabati mempunyai beberapa keunggulan dan kelemahan. Keunggulan pestisida nabati adalah : murah dan mudah dibuat sendiri oleh petani; relatif aman terhadap lingkungan; tidak menyebabkan keracunan pada tanaman; sulit menimbulkan kekebalan terhadap hama; kompatibel digabung dengan cara pengendalian yang lain; menghasilkan produk pertanian yang sehat karena bebas residu pestisida kimia. Sementara, kelemahan pestisida nabati adalah : daya kerjanya relatif lambat; tidak membunuh jasad sasaran secara langsung; tidak tahan terhadap sinar matahari; kurang praktis; tidak tahan disimpan; kadang-kadang harus disemprotkan berulang-ulang.

Tanaman yang dapat digunakan sebagai pestisida nabati antara lain tembakau (*Nicotium tabacum*), Tuba / Jenu (*Derriseleptica sp*), temu-temuan (temu hitam, kencur, kunyit), Kucai (*Allium schonaoresum*), bunga Camomil (*Chamaemelum spp*), Bawang Putih (Allium sativum), Abu Kayu, Mint (*Menta spp*), Kembang Kenikir (*Tagetes spp*), Cabai Merah (*Capsium annum*), Sedudu, Kemangi (*Ocimum sanetu*), Dringgo (*Acarus calamus*), Tembelekan (*Lantara camara*), Rumput Mala (*Artimista vulgaris*), Tomat (*Lycopersicum eskulentum*), Gamal (*Gliricidia sepium*), Bunga Mentega (*Nerium indicum*), daun Pepaya (*Caricca papaya).*

2.6 Cekaman genangan

Banjir atau genangan merupakan suatu kondisi ketika drainase berkurang atau ketika air hujan dan irigasi berlebihan. Udara pada pori tanah akan digantikan oleh air dan hal ini menghambat difusi O_2 sehingga difusi oksigen terlarut akan lebih lambat dan hanya bagian yang dekat dengan permukaan yang mengandung O_2 (Taiz & Zeiger, 2010). Hal inilah yang menyebabkan keadaan hipoksia atau bahkan anoksia di darat sehingga merugikan bagi makhluk hidup termasuk tumbuhan. Genangan sering terjadi di ekosistem dengan curah hujan yang tinggi, terutama pada tanah dengan drainase yang buruk (Visser *et al.*, 2003).

Cekaman genangan yang terjadi terbagi menjadi beberapa tingkat, mulai dari hanya tergenang pada sistem perakaran sampai terendam pada seluruh bagian tanaman. Cekaman genangan yang hanya terjadi pada sistem perakaran adalah keadaan dimana hanya pada sistem perakaran yang berada pada kondisi anaerob, sedangkan genangan yang merendam melebihi sistem perakaran terbagi menjadi dua, yaitu terendam sebagian dan terendam keseluruhan. Terendam sebagian merupakan kondisi dimana semua akar terbenam di air namun hanya sebagian batang yang tertutup oleh air. Terendam keseluruhan adalah keadaan dimana seluruh bagian tanaman terendam air (Striker, 2012).



Gambar 1. Perbedaan tingkat kelebihan air pada kapasitas lapang (Sumber : Striker & Mworia, 2012).

Genangan (*Waterlogging*) merupakan suatu keadaan dimana kelebihan air hanya berada pada pori-pori tanah atau tepat hanya pada bagian tanah saja dan dimungkinkan juga berada sangat tipis di atas permukaan tanah atau bahkan tidak sampai di atas permukaan tanah sama sekali. Oleh karena itu apabila dalam kondisi tergenang air, hanya sistem perakaran tanaman saja yang berada di bawah kondisi anaerob yang dikarenakan kekurangan oksigen sementara bagian atas tanaman tetap dalam kondisi normal atmosfer. Sedangkan terendam (*flooding submergence*) merupakan suatu keadaan dimana kelebihan air sampai ke atas permukaan tanah. Dalam kondisi terendam sebagian (*flooding partial submergence*) selain akar yang terendam, tanaman memiliki sebagian dari tunas mereka yang berada di bawah air. Sedangkan dalam kondisi terendam keseluruhan (*flooding complete submergence*), seluruh bagian tanaman berada di dalam air. Dalam kondisi seperti inilah tanaman akan menghadapi kondisi yang paling tercekam (Striker & Mworia, 2012).

Genangan menyebabkan cekaman hipoksia atau anoksia pada tanaman (Smith *et al.*, 2010). Tanaman yang tergenang dalam waktu singkat akan mengalami kondisi hipoksia (kekurangan O_2). Hipoksia biasanya terjadi jika hanya bagian akar tanaman yang tergenang (bagian tajuk tidak tergenang) atau tanaman tergenang dalam periode yang panjang tetapi

akar berada dekat permukaan tanah. Jika tanaman tergenang seluruhnya, akar tanaman berada jauh di dalam permukaan tanah dan mengalami penggenangan lebih panjang sehingga tanaman berada pada kondisi anoksia (keadaan lingkungan tanpa O_2). Kondisi anoksia terjadi pada 6-8 jam setelah penggenangan, karena O_2 terdesak oleh air dan sisa O_2 dimanfaatkan oleh mikroorganisme lain. Pada kondisi tergenang, kandungan O_2 yang tersisa dalam tanah lebih cepat habis bila terdapat tanaman karena laju difusi O_2 di tanah basah 10.000 kali lebih lambat dibandingkan dengan di udara (Dennis *et al.*, 2000). Kondisi hipoksia atau anoksia tidak hanya menghalangi fiksasi N, tetapi juga distribusi N dan mineral lain sehingga menghambat pertumbuhan akar dan nodulasi. Akibat transportasi N dan mineral ke bagian tajuk tidak mencukupi, daun akan menguning yang akan diikuti oleh pengguguran daun. Scott *et al.*. (1989) melaporkan, pengaruh penggenangan ditunjukkan oleh daun yang menguning, pengguguran daun pada buku terbawah, kerdil, serta berkurangnya berat kering dan hasil tanaman.

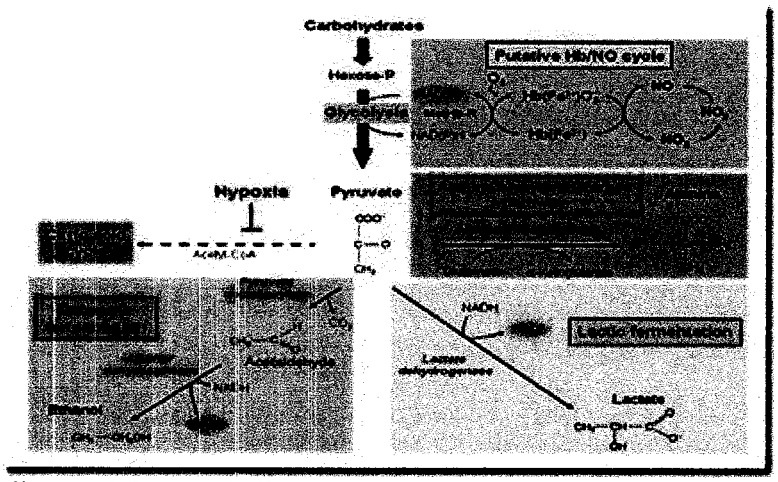
Gejala kerusakan pada tumbuhan yang timbul akibat genangan antara lain layu, penuaan daun, dan epinasti (Marschner, 1995). Selain itu, genangan menyebabkan gangguan penyerapan hara oleh akar akibat rendahnya ATP yang dihasilkan melalui proses respirasi dan rendahnya permeabilitas akar menyebabkan menurunnya potensial air pada daun yang selanjutnya menyebabkan layu. Daun tua akan mengalami penuaan karena alokasi hara ke daun yang lebih muda. Gejala-gejala kerusakan fungsi sel akar yang timbul akibat genangan dapat menganggu proses pertumbuhan dan perkembangan tumbuhan yang akan mempengaruhi hasil (*yield*) (Taiz & Zeiger, 2010).

2.7 Respon dan adaptasi molekuler tanaman terhadap cekaman genangan

Genangan menyebabkan cekaman hipoksia atau anoksia pada tanaman (Smith *et al.*, 2010). Cekaman genangan menyebabkan peningkatan kerapatan tanah, fitotoksik dari produk samping, penurunan pH, dan penurunan oksigen (Parent *et al.*,., 2008). Penurunan pH merupakan penyebab utama terjadinya kerusakan dan seringkali menyebabkan kematian sel pada tanaman yang sensitif (Hodson & Bryant, 2012). Rendahnya ketersediaan O_2 di tanah akibat genangan akan mengubah aktivitas respirasi sel dari aerob menjadi anaerob. Pada respirasi anaerob, ATP dan NAD^+ dihasilkan dari glikolisis dan sangat tidak efektif karena fermentasi hanya menghasilkan 2-4 mol ATP untuk tiap mol heksosa. Fermentasi yang terjadi saat keadaan anaerob yaitu fermentasi laktat dan etanol. Saat anaerob, piruvat yang dihasilkan dari glikolisis tidak diubah menjadi asetil CoA tetapi piruvat diubah menjadi laktat

oleh laktat dehidrogenase (LDH). Fermentasi laktat menyebabkan akumulasi laktat sehingga pH sitosol menjadi rendah. Enzim LDH menjadi tidak aktif akibat rendahnya pH sitosol. Hal ini menyebabkan perubahan fermentasi dalam sel dari fermentasi laktat menjadi fermentasi etanol. Akumulasi etanol merupakan stategi untuk bertahan pada keadaan tergenang karena mencegah penurunan pH sitosol dengan cara menghambat aktivitas LDH dan mengaktifkan piruvat dekarboksilase (PDC) yang menghasilkan asetaldehid dan selanjutnya asetaldehid diubah menjadi etanol oleh alkohol dehidrogenase (ADH) (Pucciariello & Perata, 2012). Akumulasi ini merupakan hasil dari peningkatan aktivitas ADH (Marschner, 1995).

Selain laktat dan etanol, asam amino seperti alanin dan gama amino butyric acid (GABA) juga terlibat dalam strategi pertahanan tumbuhan terhadap genangan. Alanin dan GABA berperan dalam memperkecil penurunan pH sitosol (Pucciariello & Perata, 2012). Strategi lain bertahan pada genangan yaitu detoksifikasi ion Fe2+ dengan aktivitas SOD, meningkatnya aktivitas sitokrom oksidase yang memiliki afinitas tinggi terhadap O_2, dan mencegah rendahnya pH sitosol (Marschner, 1995). Jalur metabolisme tumbuhan saat tergenang disajikan pada Gb 3.



Gambar 3. Skema diagram jalur metabolik utama yang diusulkan pada saat tanaman mengalami stres genangan. (Sumber : Parent *et al.*, 2008).

Hipoksia dan anoksia dikenali oleh suatu sensor yang mengaktifkan gen metabolisme anaerobik tertentu. Sinyal yang berhubungan dengan etilen aktif pada kondisi hipoksia. Sinyal tersebut meregulasi gen ERF yang masuk grup VII, yang menstimulasi atau menghambat jalur sinyal transduksi yang menginisiasi LOES (*Low Oxygen Escape Syndrome*) dan LOQS (*Low Oxigen quiecence Syndrome*). Anoksia mengaktifkan jalur yang berhubungan dengan ROS termasuk aktivasi *Heat Shock Factors* (HSF), yang meningkatkan transkripsi *Heat Shock Protein* (HSP) (Pucciariello & Perata, 2012). LOES dan LOQS

merupakan bentuk strategi tanaman pada kondisi tergenang. Salah satu bentuk dari sindrome LOQS adalah pengurangan pertumbuhan tunas yang tergenang untuk mempertahankan ketersediaan substrat hingga genangan surut. Sedangkan LOES ditunjukkan dengan tumbuh cepatnya tunas yang tergenang untuk mencapai permukaan air dan mengatur pertukaran gas (Colmer & Voesenek, 2009).

Sintesis etilen membutuhkan oksigen, sehingga dapat terjadi dalam keadaan hipoksia dan tidak dapat terjadi dalam kondisi anoksia (Videmsek dkk., 2006). Etilen memulai dan mengatur beberapa adaptasi molekular, respon kimia, dan morfologi yang memungkinkan tanaman untuk menghindari anaerobiosis dengan meningkatkan ketersediaan oksigen ke akar pada kondisi tanah yang tergenang, seperti pengembangan aerenkim (Sairam dkk., 2008). Peningkatan etilen sejalan dengan peningkatan lama genangan yang mengindikasikan adanya oksigen yang merubah ACC (*1-Aminocyclopropane-1-Carboxylate*) menjadi etilen. Menurut Bradford (2008), ACC merupakan prekusor etilen, perubahan ACC menjadi etilen tergantung ketersesdiaan oksigen dan dikatalis oleh enzim ACC oksidase. Saat terjadi genangan, konsentrasi etilen akan meningkat karena etilen tidak berdifusi keluar dari akar (Smith *et al.*, 2010). Hasil penelitian Peeters *et.al.*, (2002), pada tanaman *Rumex palustri* menunjukkan bahwa genangan selama satu jam mengakibatkan peningkatan konsentrasi etilen 20 kali dari 0.05 μL^{-1} menjadi 1.0 μL^{-1} dibandingkan yang tidak tergenang.

Pada dasarnya, akumulasi etilen yang meningkat di bawah kondisi hipoksia menyebabkan regulasi tingkat ABA menurun melalui penghambatan ekspresi 9-cis-epoxycarotenoid dioxygenase / NCED (salah satu enzim yang digunakan untuk mengubah 9-*cis-violaxanthin* dan 9-*cis-neoxantin* menjadi prekusor ABA yaitu Xanthoxin). Selain itu, penghambatan lain melalui aktivasi pemecahan ABA menjadi bentuk intermediet ABA yang tidak stabil yaitu 8'-hydroxy ABA oleh ABA 8'hydroxyilase (ABA8ox) (Cluter & Krochko, 1999), yang secara langsung dikonversi ke bentuk *phaseic acid* (PA) dari proses isomerase (Endo *et al.*, 2014). PA dapat direduksi menjadi bentuk tidak aktif *Dihydrophaseic acid* (DIPA). Hal ini dapat menyebabkan penurunan kandungan ABA endogen. Di sisi lain, ABA dapat dinonaktifkan melalui konjugasi dengan glukosa. Proses Glukosilasi pada gugus karboksil ABA dikatalisis oleh *glucosyltransferase* untuk mengubah ABA menjadi bentuk ABA-GE (ABA glucose ester). Penurunan kandungan ABA endogen pada internode ini dibutuhkan untuk menstimulasi ekspresi (GA) 3-oxidase (sebuah enzim yang mengakatalis perubahan menjadi giberelin bioaktif /GA1) (Salazar *et al.*,2015). Di samping itu, peningkatan hormon etilen endogen akan menghasilkan pH yang lebih rendah dalam apoplas

yang membantu merangsang pelonggaran dinding sel sebagaimana tahapan yang dibutuhkan untuk mengawali pemanjangan sel (Jackson, 2003).

BAB 3. TUJUAN DAN MANFAAT PENELITIAN

3.1 Tujuan Khusus Penelitian

1. Mempelajari karakter molekuler tanaman tembakau yang toleran pada cekaman genangan secara periodik sebagai kandidat bahan baku bioinsektisida
2. Mempelajari karakter morfologi dan fisiologi tanaman uji yang diberi aplikasi bioinsektisida tanaman tembaku toleran pada cekaman genangan secara periodik

3.2 Tujuan Umum Penelitian

Mengkaji prospek tanaman tembakau yang toleran genangan sebagai kandidat bahan baku bioinsektisida dengan memperhatikan karakter morfologi dan fisiologi serta molekuler tanaman.

3.3 Manfaat Penelitian

1. Memberikan kontribusi bagi program pemuliaan tanaman dengan menyedikan sumber genetik tanaman tembakau yang toleran terhadap genangan dan banjir
2. Diversifikasi pemanfaatan tembakau sebagai bahan bioinsektisida selain untuk bahan baku rokok.
3. Membantu masyarakat mengatasi permasalahan budidaya tanaman tembakau yang sensitive terhadap musim/cuaca, khususnya musim penghujan.

BAB 4. METODE PENELITIAN

4.1 Lokasi Penelitian

Penelitian dilaksanakan di rumah kaca kebun percobaan PT Sadhana di Purwoasri Pasuruan Jawa Timur dan Laboratorium Biosains dan Tenologi Tumbuhan Jurusan Biologi FMIPA ITS.

4.2 Sampel Penelitian

Varietas tanaman tembakau yang terpilih sebanyak 5 macam adalah jepon mawar, srumpung, jepon palakean dan sumporis. Tanaman terpilih tersebut kemudian ditanam sesuai dengan tahap pelaksanaan penelitian.

4.3 Pelaksanaan Penelitian

Tahapan pelaksanaan penelitian berupa:

4.3.1 Persiapan Benih

Benih yang digunakan terdiri dari 4 varietas berbeda yaitu Marakot, Jinten, Jepon Pelakean, dan Manilo. Dilakukan penimbangan benih seberat 0,05 gram/nampan untuk masing-masing varietas. Benih direndam dalam larutan GA3 100 ppm secukupnya selama ± 24 jam untuk mempercepat perkecambahan, lalu ditiriskan selama ±2 hari.

4.3.2 Penyebaran Benih

Penyebaran benih dilakukan dalam nampan dengan campuran media *vermicompos* dan *coco peat* (1:1). Setiap nampan membutuhkan campuran kedua media tersebut sebanyak 1 liter media. Kemudian ditambahkan pupuk NPK. (5 gr/lt media) dan disiapkan larutan ridomil (0,5 gram/liter air). Semua bahan dicampur rata, dan larutan ridomil (antifungal) ditambahkan perlahan hingga media cukup lembap. Setelah media tercampur rata, kemudian diisikan pada nampan hingga tinggi media ± 2 cm. Sebelumnya, masing-masing nampan diberi label nama varietas dan tanggal penyebaran benih. Pengisian media pada nampan cukup disebar rata saja, tanpa dipadatkan. Sebelum penyebaran benih dilakukan, media diletakkan pada nampan yang telah berisi air setinggi ± 1 cm selama beberapa saat hingga media lembap, lalu nampan diangkat dan ditiriskan. Diambil segenggam tanah kompos yang telah diayak halus, kemudian dicampur dengan benih yang telah ditiriskan dari larutan GA_3. Penyebaran benih dilakukan dengan pola seperti gambar 3.3 (A). Setelah benih tersebar merata, kemudian nampan ditutup dan disimpan di

dalam ruangan. Pengecekan rutin per hari dilakukan terhadap jumlah air pada nampan dan kondisi perkembangan benih hingga terjadi pecah benih (berkecambah) yang ditandai berubahnya warna benih menjadi putih di permukaan media. Lalu, penutup nampan dibuka, ditiriskan, dan dikeluarkan ke para-para. Setiap pukul 10.00 WIB, para-para ditutup dengan para *net* dan dilakukan pengembunan dengan *sprayer* setiap 1 jam sekali (menyesuaikan kondisi cuaca).

Setiap hari dicek kondisi media, jika media kering maka nampan diletakkan kembali pada nampan bagian bawahnya yang telah berisi air (tidak sampai menggenangi permukaan media) selama beberapa saat. Setelah tanah kembali lembap, nampan ditiriskan kembali. Proses perkecambahan benih pada nampan dilakukan selama 15-20 HSS (Hari Setelah Sebar) hingga memenuhi kriteria *pricking*, yaitu munculnya 3-4 daun.

4.3.3 *Pricking*

Pricking dilakukan dalam tray dengan 72 lubang (6x12). Komposisi media tray sama dengan media dengan media untuk sebar benih pada nampan, yaitu campuran media *vermicompos* dan *coco peat* (1:1). Media yang dibutukan untuk tiap tray sebanyak ± 2 liter media. Kemudian ditambahkan pupuk NPK (5 gr/lt media) dan disiapkan larutan ridomil (0,5 gram/liter air). Semua bahan dicampur rata, dan larutan ridomil ditambahkan perlahan hingga media cukup lembap. Pricking dilakukan pada bibit yang telah memenuhi kriteria, yaitu munculnya 3-4 daun. Tray yang akan digunakan untuk pricking, disemprot air hingga ada air menetes dari lubang bagian bawah tray. Proses pricking dilakukan dengan alat pinset steril (dicelupkan dalam larutan sabun) untuk mengambil benih dari nampan dan kayu untuk membuat lubang tanam pada media tray. Bibit dicabut perlahan (dijepit dengan pinset di bagian daun), sehingga atidak merusak akar. Lalu, bibit dimasukkan ke dalam lubang tanam yang telah dibuat dan lubang ditutup kembali dengan media.

Setelah proses pricking selesai, tray diletakkan dalam ruangan selama ±3 hari. Setelah masa adpatasi selesai, tray dapat dikeluarkan ke para-para yang ditutup dengan para-net. Sama seperti pemeliharaan benih pada nampan, yaitu dilakukan pengembunan setiap satu jam sekali (menyesuaikan kondisi cuaca).

Daun tembakau yang telah mencapai ukuran sebesar uang koin 500 rupiah, maka dilakukan *clipping* menggunakan silet steril. Hal tersebut untuk memperkokoh batang maupun akar dan memeratakan pertumbuhan. Setelah *clipping*, tidak boleh dilakukan penyiraman hingga hari berikutnya. Pertumbuhan bibit pada tray dilakukan selama 20-25 hari untuk kemudian penanaman pada polybag.

4.3.4 Pemindahan pada *Polybag*

Pembuatan media *polybag* (dalam hal ini *polybag* yang dimaksudkan adalah plastik berukuran 3 kg dan ketebalan 0,5 mm yang diberi lubang sendiri) menggunakan campuran antara media kompos dan arang sekam (2:1). Keduanya dicampur hingga rata. Total polybag yang digunakan adalah 40 buah, dimana tiap varietas 10 *polybag* (@5 *polybag* untuk perlakuan dan kontrol) . Setiap *polybag* berisi satu tanaman dengan tinggi media ± 16 cm. Pada *polybag* yang telah diberi label varietas, disiram hingga membasahi seluruh media, kemudian dibuat lubang tanam pada bagian tengah media *polybag* menggunakan kayu/bambu.

Pemindahan bibit dari tray ke *polybag* dengan mencabut bibit yang ukurannya seragam menggunakan tangan pada bagian daun (bukan batang), sambil menekan bagian bawah media *tray*. Bibit dimasukkan pada lubang tanam, kira-kira bibit tidak goyah dan lubang tanam ditutup kembali dengan media.

Bibit yang baru saja ditanam kemudian diberi air secukupnya (dituang). Setelah semua bibit selesai ditanam, dibuat kembali lubang agak jauh dari bibit untuk tempat masuknya pupuk awal NPK sebanyak 3 gram/*polybag*. Kemudian dilakukan pengecekan pH tanah menggunakan *soil tester* agar sesuai dengan pH optimal bagi pertumbuhan tembakau yaitu 5,5 – 6,8 (Matnawi,1998).

Selama pertumbuhan pada *polybag*, dilakukan dua macam aklimatisasi, yaitu ±7 hari tanpa penyiraman dan selanjutnya dengan penyiraman hingga bibit berumur 60-70 HSS (dosis penyiraman 50 ml air/*polybag* setiap hari). Selama aklimatisasi dilakukan sekali penyemprotan insektisida organtrin dengan dosis 1,5 ml/lt air. Penyemprotan dilakukan dengan *sprayer*.

4.3.5. Pemberian Cekaman Genangan

Bibit yang telah mencapai umur 60-70 HSS dengan moroflogi 4-5 daun, kemudian dilakukan cekaman genangan dalam *container plastik* berukuran 40 cm x 30 cm x 20 cm. Setiap *container* diisi 3 polybag untuk varietas yang sama. Setiap *container* diberi label untuk pengukuran tinggi air dan label nama varietas. Perlakuan genangan tahap pertama dengan mengisi air pada *container* setinggi 13 cm hingga tercapainya kondisi bagian akar terendam dan tanah tersaturasi oleh air (*soil waterlogging*) selama 5 hari. Ketinggian air harus terus dijaga tetap 13 cm.

Perlakuan genangan tahap kedua dilakukan penambahan air pada *container* hingga merendam sebagian batang dan 1-2 daun pertama (*partial submergence*). Tahap kedua ini juga dilakukan selama 5 hari, sehingga total pemberian cekaman genangan selama 10 hari.

4.4 Persiapan larva

Larva *Plutela xylostella* didapatkan dari Balittas Malang dan dimasukkan ke dalam toples, toples lalu ditutup dengan kain putih dan diikat dengan karet. Telur dipelihara hingga berubah menjadi larva instar I, makanan yang diberikan untuk pemeliharaan larva ini adalah daun kailan segar yang diganti setiap hari serta kotorannya dibersihkan dengan kuas sehingga memasuki instar III yang siap untuk digunakan sebagai larva uji (Arivoli dan Tennyson, 2013). Dalam arifin (1990) menyebutkan bahwa instar III- IV merupakan fase yang paling banyak menyerang dimana larva ini dapat memakan seluruh daun sampai ketulang-tulang daunnya sehingga akan sangat mengganggu pertumbuhan tanaman yang diserangnya. Dengan adanya hal tersebut maka digunakan larva instar III.

4.5 Pembuatan Ekstrak Daun Tembakau

Pembuatan ekstrak daun tembakau (*Nicotiana tabacum*) dibuat dengan menggunakan metode maserasi atau perendaman dengan beberapa modifikasi. Langkah awal dimulai dengan mengambil daun tembakau kemudian dibersihkan dengan akuades dan dikering-anginkan tanpa terkena sinar matahari langsung pada suhu kamar. Setelah kering, daun dipotong kecil-kecil dan dihaluskan menggunakan blender. Hasil dari blenderan dikeringkan dalam suhu ruang, setelah kering ditimbang beratnya. Kemudian hasil blenderan di maserasi dalam etanol 96% dengan perbandingan 1:5 (10 gram serbuk dengan 50 ml etanol) (Zuhrotun *et al*, 2010). Perendaman (Maserasi) dilakukan pada suhu kamar hingga 72 jam. Proses perendaman ini bertujuan untuk meluruhkan seluruh kandungan senyawa bioaktif yang terkandung dalam daun tersebut agar dapat tertarik keluar. Proses maserasi menggunakan konsep senyawa polar menarik senyawa polar dan sebaliknya, serta senyawa organic menarik senyawa organic dan sebaliknya (Lechninger,1982). Setelah 72 jam, hasil maserasi disaring dengan corong *buchner* yang dialasi kertas saring, selanjutnya hasil ekstraksi diuapkan dengan menggunakan *rotary evaporator* sampai dihasilkan ekstrak murni daun tembakau

(Dadang dan Kanju, 2000). Ekstrak daun tembakau tersebut disimpan di lemari es sampai saat digunakan untuk pengujian.

Hasil ekstrak daun tembakau diencerkan dengan akuades dan dibantu dengan beberapa tetes pengemulsi DMSO serta etanol dan diperoleh konsentrasi 10 %, 20 %, 30%, 40 %, 50 %, 60 %, 70 %, 80 % dan 90 %. Sedangkan sebagai kontrol digunakan akuades dengan tambahan beberapa tetes DMSO dan etanol.

4.6 Uji Pengaruh Ekstrak Daun Tembakau Tehadap Larva

Uji pengaruh ekstrak daun tembakau terhadap mortalitas larva *Plutela xylostella* bertujuan untuk melihat pada konsentrasi berapa saja yang mampu menjadi ekstrak yang toksik terhadap larva, penentuan menentukan nilai LC50 dari ekstrak daun tembakau yang dapat mematikan 50% dari larva uji yang hidup, perhitungan mortalitas, persentase pembentukan pupa, serta untuk data uji antifeedant sebanyak 3 kali ulangan. Ekstrak terdiri dari 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% sebanyak 3 kali ulangan.

Langkah awal yang dilakukan dalam uji ini ialah disiapkan larva *Plutela xylostella* yang sudah instar III dimasukkan ke toples dengan masing-masing toples 20 ekor larva. Pengujian menggunakan metode residu. Daun sawi segar dipotong dan ditimbang dan dilakukan pencelupan potongan daun sawi ke dalam ekstrak daun tembakau dalam berbagai konsentrasi yang sudah disiapkan sesuai dengan uji yang dibutuhkan hingga basah merata, lalu dikeringanginkan dengan berat daun 0.5 gram yang digunakan sebagai makanan larva. (Harwanto et al, 2012). Makanan diberikan tiap 24 jam sekali. Pemberian daun sawi segar yang dicelupkan kedalam ekstrak daun tembakau dilakukan dan diganti setiap 24 jam hingga mencapai 72 jam. Pengamatan dilakukan hingga mencapai masa pupa untuk data perkembangan.

4.7 Uji Antifeedant

Pengujian antifeedant bertujuan untuk melihat kandungan senyawa antifeedant dari daun tembakau terhadap larva *Plutela xylostella* yang dilakukan dengan metode residu pada daun. Aktifitas antifeedant suatu ekstrak tanaman dinilai dengan berdasarkan indeks antifeedant. Dimana semakin tinggi suatu kandungan antifeedant menyebaban penurunan aktifitas makan larva uji. Dalam penelitian sebelumnya disebutkan bahwa aktifitas antifeedant dari masing-

masing ekstrak tanaman bervariasi tergantung pada jenis pelarut yang digunakan dalam proses ekstraksi (Arivoli dan Tennyson, 2013).

Pengujian antifeedant sejalan dengan uji pengaruh ekstrak daun tembakau yaitu daun perlakuan pada hari pertama. Daun sawi segar dipotong dan ditimbang serta dilakukan pencelupan potongan daun sawi ke dalam ekstrak daun tembakau dalam berbagai konsentrasi yang sudah disiapkan sesuai dengan uji yang dibutuhkan hingga basah merata, lalu dikeringanginkan dengan berat daun 0.5 gram yang digunakan sebagai makanan larva. Pengaruh penghambatan antifidant dihitung dengan menggunakan rumus :

$$AF = \left(\frac{K-P}{K+P}\right) \times 100\%$$

Keterangan :

AF : antifidant

P : Berat daun yang dimakan pada kontrol

K : Berat daun yang dimakan pada perlakuan

(Harwanto et al, 2012).

Data hasil Antifeedant kemudian dianalisis dengan anova yang dilanjut dengan uji Duncan.

4.8 Rancangan Penelitian

Rancangan yang digunakan dalam penelitian ini adalah rancangan acak lengkap dengan 3 kali ulangan. Konsentrasi ekstrak yang digunakan adalah 9 konsentrasi ekstrak daun tembakau dan 1 kontrol, kemudian dari 10 konsentrasi yang akan diujikan selama 3 hari dicari nilai ambang batas bawah LC10 dan ambang batas atas LC90 serta LC_{50}, pengamatan pola mortalitas, persentase pembentukan pupa serta uji kandungan antifeedant.

4.9 Analisis Data

Analisis data dilakukan dengan hipotesis sebagai berikut :

Ho : ekstrak daun tembakau tidak berpengaruh sebagai bioinsektisida terhadap larva *Plutela xylostella*

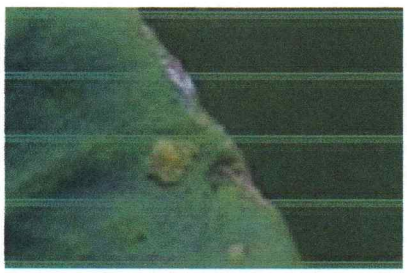
Hi : ekstrak daun tembakau berpengaruh sebagai bioinsektisida terhadap larva *Plutela xylostella*

Untuk mengetahui pengaruh perlakuan pada parameter yang diamati yaitu mortalitas, perkembangan serta kandungan antifeedant dilakukan analisis probit dengan menggunakan minitab, serta uji statistic ANOVA one way dengan taraf kepercayaan 95%. Setelah itu dilakukan uji lanjutan DMRT untuk membandingkan perlakuan yang paling efektif antara tiap-tiap perlakuan (Utami, 2010).

BAB 5. HASIL DAN LUARAN YANG DICAPAI

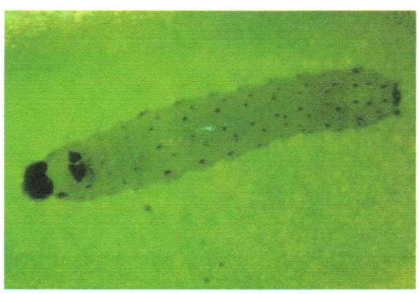
5.1 Daur Hidup dan Morfologi *Plutella xylostella*

Menurut Sastrosiswojo, *dkk.*, (2005), *P. xylostella* memiliki telur berbentuk oval dan pipih dengan ukuran 0,6 mm x 0,3 mm. Warna telur yaitu kuning pucat atau hijau pucat, berkilau, dan lembek. Biasanya dalam satu ngengat betina dapat bertelur sekitar 250-300 butir, namun rata-rata produksi telur ngengat betina adalah 150 butir dan ngengat betina bertelur selama 5-6 hari (Capinera, 2015).



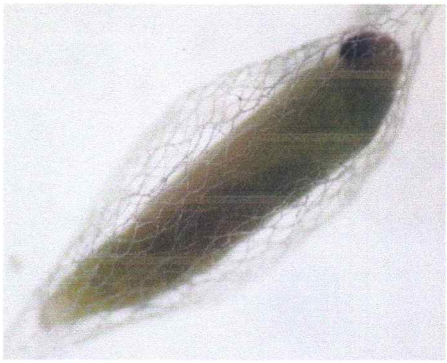
Gambar 5.1 Telur *Plutella xylostella* (Hermansson, 2016)

Larva *P. xylostella* memiliki bentuk silindris, berwarna hijau muda, tidak berbulu dan mimiliki lima pasang *proleg*. Larva tersebut terdiri atas empat instar, panjang stadium larva instar satu adalah 4 hari, larva instar kedua 3 hari, larva instar ketiga 3 hari dan larva keempat 4 hari (Sastrosiswojo, *dkk.*, 2005). Fase larva pada umumnya menyerang tanaman muda, namun terkadang dapat pula merusak tanaman yang sedang dalam pembentukan bunga. Larva yang baru menetas akan merayap pada permukaan daun, melubangi epidermis dan memakan permukaan bagian bawah daun sehingga hanya tersisa tulang-tulang daun (Winarto dan Darmawati, 2004).



Gambar 5.2 Larva *Plutella xylostella* (Dokumentasi pribadi) (Perbesaran 3x)

Masa pupa *P. xylostella* terjadi rata-rata selama 8,5 hari (Carpinera, 2015). Pupa *P. xylostella* terjadi antara larva instar keempat, yang mana panjang pupa rata-rata 6,3-7,0 mm dan lebar pupa adalah 1,5 mm. Pupa *P. xylostella* terbungkus oleh kongkon (jala sutera) dan diletakkan pada permukaan daun (Sastrosiswojo, *dkk.*, 2005).



Gambar 5.3 Pupa *Plutella xylostella* (Dokumentasi pribadi) (Perbesaran 2,5x)

Ngengat dari *P. xylostella* memiliki panjang sekitar 6 mm yang memiliki warna coklat kelabu, aktif pada malam hari dan mudah terbawa oleh angin (Capinera, 2015). Sayap *P. xylostella* memiliki tiga buah lekukan (undulasi) yang berwarna putih menyerupai berlian, oleh karena itu *P. xylostella* biasa disebut dengan Diamondback Moth.

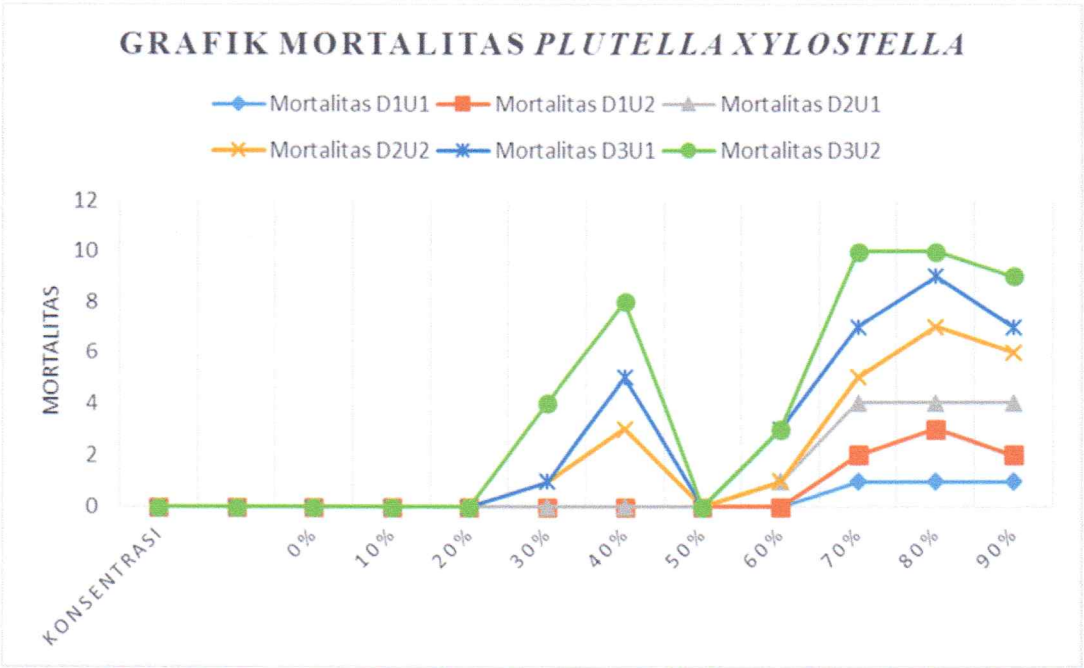
Gambar 5.4 Imago *Plutella xylostella* (Capinera, 2015)

5.2 Pengaruh Ekstrak Daun Tanaman Tembakau Terhadap Mortalitas Larva *Plutela xylostella*

Penelitian ini bertujuan untuk mengetahui efek toksisitas dari ekstrak daun tembakau pada cekaman genangan secara periodic terhadap pola mortalitas larva *Plutela xylostella* yang berlangsung selama 72 jam. Perlakuan dilakukan dengan berbagai macam konsentrasi mulai dari 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% serta kontrol sebanyak 3 kali ulangan. Kemudian dilakukan analisis probit untuk mencari nilai LC50 dan uji anova untuk mengetahui pengaruh ekstrak daun tembakau terhadap mortalitas larva *Plutela xylostella.*

Hasil analisis probit tersebut ditampilkan pada Gambar 1. Berdasarkan grafik mortalitas pada Gambar 1, mortalitas *Plutella xylostella* dengan perlakuan pemberian ekstrak daun tembakau mengalami kematian hari pertama mulai dari konsentrasi 70%, 80% dan 90%, pada hari kedua dan ketiga *Plutella xylostella* mengalami kematian pada konsentrasi 30 %, 40 %, 60%, 70%, 80%, 90%. Kematian yang terjadi dimulai dari konsentrasi 30% dikarenakan

bahwa ekstrak daun tembakau dibawah konsentrasi 30% tidak efektif untuk membunuh *Plutella xylostella* karena kandungan senyawa pada tanaman *Nicotiana tabacum* semakin kecil konsentrasi maka kandungan senyawa aktifnya juga semakin sedikit, akibatnya tidak memiliki pengaruh terhadap kematian *Plutella xylostella* dan sebaliknya semakin tinggi konsentrasi filtrat maka kandungan senyawa metabolitnya semakin banyak maka kandungan bahan aktif dalam larutan juga semakin banyak sehingga daya racun dari biopestisida nabati semakin tinggi (Afifah, *dkk.*, 2015).

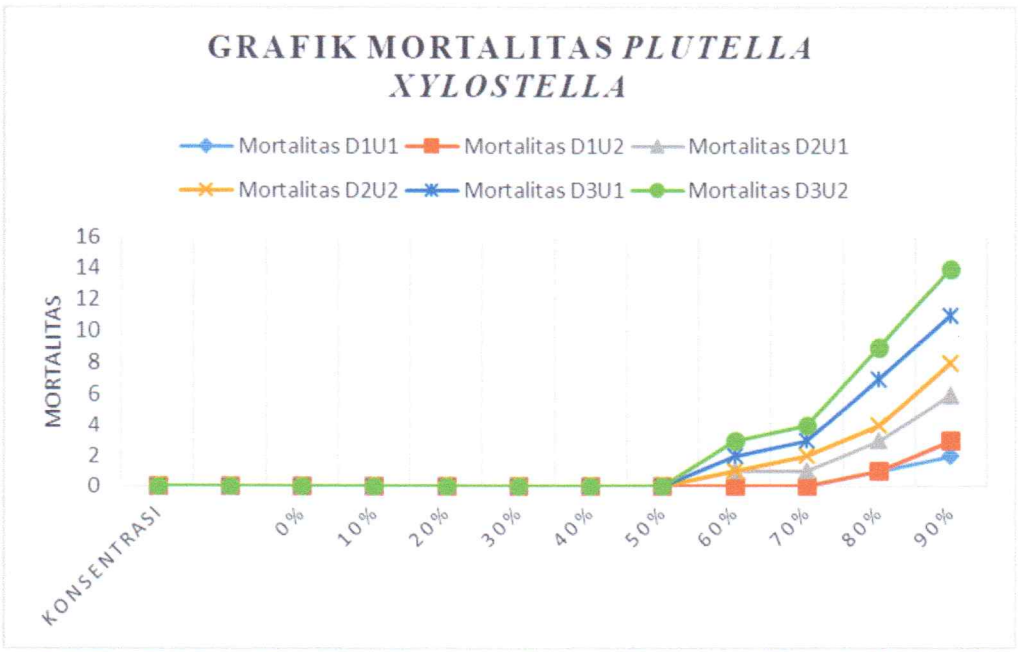


Gambar 5.5 Grafik Mortalitas *Plutella xylostella* pada Perlakuan Ekstrak Daun Tembakau

Plutella xylostella yang mengalami kematian disebabkan oleh masuknya pestisida nabati yaitu ekstrak daun tembakau (*Nicotiana tabacum*) kedalam tubuh serangga sebagai racun perut yang masuk melalui saluran pencernaan makanan yang selanjutnya akan menembus dinding usus dan akan mengakibatkan terganggunya metabolisme serangga sehingga akan terjadi kekurangan energi yang diperlukan untuk aktivitas hidup serangga, serta akan terjadi kejang-kejang dan lambat laun akan menyebabkan kematian (Safirah, *dkk.*, 2016).

Gambar 5.6 Kematian *Plutella xylostella* pada Perlakuan Ekstrak Daun *Nicotiana tabacum*

Serangga yang menyerap senyawa yang ada pada pestisida nabati misalnya terpenoid yang memiliki rasa pahit dan bersifat *antifeedant* akan menghambat aktivitas makan serangga. Triterpenoid juga bersifat penolak serangga (*repellent*) yang mana senyawa ini akan masuk ke dalam saluran pencernaan melalui makanan yang serangga makan, kemudian diserap oleh saluran pencernaan tengah dan saluran tersebut berfungsi sebagai tempat perombakan makanan secara enzimatis sehingga senyawa triterpenoid sebagai racun perut yang dapat mematikan serangga (Afifah, *dkk*., 2015)



Gambar 5.7 Grafik Mortalitas *Plutella xylostella* pada Perlakuan Ekstrak Batang Tembakau

Berdasarkan grafik mortalitas pada Gambar 5.7, mortalitas *Plutella xylostella* dengan perlakuan pemberian ekstrak batang tembakau mengalami kematian hari pertama mulai dari konsentrasi 80% dan 90%, pada hari kedua dan ketiga *Plutella xylostella* mengalami

kematian pada konsentrasi 60%, 70%, 80% dan 90%. Kematian yang terjadi dimulai dari konsentrasi 80% dikarenakan semakin tinggi konsentrasi ekstrak batang tembakau maka kandungan senyawa aktifnya juga semakin tinggi sehingga daya racun dari biopestisida nabati semakin tinggi (Afifah, *dkk.*, 2015).

Berdasarkan hasil tersebut dapat dikatakan bahwa nikotin bersifat racun kontak, yang artinya memiliki sifat yang mematikan secara langsung. Mekanisme racun kontak tersebut adalah nikotin akan diserap oleh tubuh melalui kulit sehingga akan mengganggu sistem pernafasan yang mengakibatkan ulat susah bernafas dan menyebabkan mortalitas (Kardinan, 2004).

Plutella xylostella mengalami kematian setelah diberikan perlakuan ekstrak batang tembakau dikarenakan ekstrak batang tembakau masuk ke dalam organ pencernaan serangga melalui pakan yang yang dicelupkan ke dalam ekstrak batang tembakau. Selanjutnya akan dibawa oleh cairan tubuh serangga ke susunan syaraf serangga (Habieb, 2016).

Nikotin merupakan senyawa aktif yang dapat membunuh serangga. Kardinan (2004) mengatakan bahwa nikotin bekerja sebagai fumigan yang akan menguap dan menembus secara langsung ke integumen ulat dan akan menyerang sistem pernafasan ulat. Nikotin akan masuk ke dalam tubuh ulat melalui spirakel dalam sistem trakea dan menyebabkan sistem syaraf ulat terganggu. Selain senyawa nikotin yang menyebabkan mortalitas pada *Plutella xylostella* adalah senyawa terpenoid. Senyawa terpenoid memiliki rasa pahit sehingga menghambat aktivitas makan ulat.

5.3 Luaran Yang Dicapai

No	Jenis Publikasi	Rencana Submit	Keterangan
1	Jurnal Internasional	Particular variety of tobacco Nicotiana tabacum) exhibits distinct morphological and physiological responses against periodic waterlogging stress To cite this article: *Journal Physic Conf. Ser.* **1028** 012035	Sudah Terpublish
2	Jurnal Internasional	Data Anatomical Response of Roots Variety of Tobacco Plants (Nicotiana tabbacum) In Periodic waterlogging	Sudah Terpublish

		Stress : ***Data in Brief Elsevier***	
2	Seminar Internasional	IBOC di Jurusan Biologi FIA ITS Tanggal 10 Oktober 2018	Sudah Dilaksanakan
3	Produk Bioinsektisida		Ekstrak Daun Tembakau

BAB 6. RENCANA TAHAPAN BERIKUTNYA

Rencana pada penelitian berikutnya perlu dilakukan uji multilokasi dan uji hetero tanaman untuk mengetahui daya pestisida di lingkungan makro. Untuk hetero tanaman ini juga dibedakan pada tanaman pangan dan tanaman non pangan.

BAB 7. KESIMPULAN DAN SARAN

7.1 Kesimpulan

1. Ekstrak daun Tembakau berpotensi sebagai insektisida nabati.
2. Ekstrak daun tembakau berpengaruh terhadap mortalitas *Plutella xylostella* instar 3 dan didapatkan LC50 didapatkan pada konsentrasi 50% dari ekstrak daun.
3. Ekstrak batang tembakau berpengaruh terhadap mortalitas *Plutella xylostella* instar 3 dan didapatkan LC50 didapatkan pada konsentrasi 80% dari ekstrak batang.

7.2 Saran

1. Perlu dilakukan penelitian skala rumah kaca atau aplikasi langsung ke lahan pertanian agar lebih aplikatif
2. Dalam pemeliharaan kontrol larva *S. litura* agar dapat menjadi pupa perlu diperhatikan faktor-faktor lingkungan meliputi suhu dan kelembabannya serta kebersihan tempat pemeliharaan.

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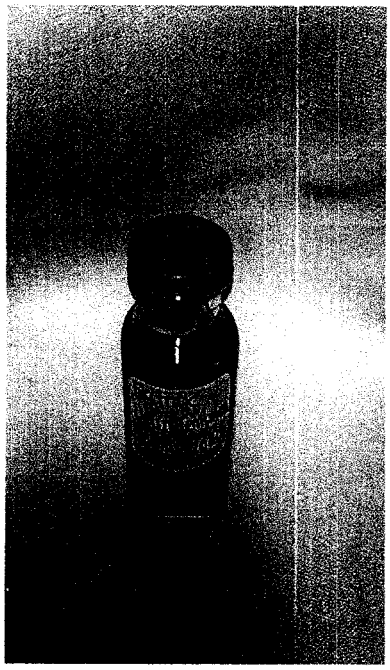
TEKNOLOGI TEPAT GUNA



- Ekstrak daun Tanaman Tembakau sebagai bahan dasar biopestisida ramah lingkungan
- Konsentrasi 40% sudah menyebabkan mortalitas pada Spodoptera litura pada
- Teknologi Tepat Guna :
  - Berupa cairan
  - Cairan Berwarna Hijau Tua
  - Bau Khas Tembakau

FORMULIR EVALUASI ATAS CAPAIAN LUARAN KEGIATAN

Ketua : Drs HERY PURNOBASUKI M.Si, Ph.D

Perguruan Tinggi : Universitas Airlangga

Judul : Pemanfaatan Tanaman Tembakau (Nicotiana tabacum) Toleran Genangan Pada Cekaman Genangan Secara Periodik Sebagai Bahan Bioinsektisida Yang Ramah Lingkungan

Skema : Penelitian Terapan Unggulan Perguruan Tinggi

Waktu Kegiatan : Tahun ke 2 dari rencana 2 tahun

LUARAN YANG DIRENCANAKAN DAN JUMLAH CAPAIAN

No	Luaran yang Direncanakan	Jumlah Capaian
1	Publikasi ilmiah	3
2	*Keynote speaker dalam pertemuan ilmiah*	4
3	Karya Tulis Ilmiah	1

CAPAIAN DISERTAI DENGAN LAMPIRAN BUKTI-BUKTI LUARAN KEGIATAN

1. PUBLIKASI ILMIAH

	Keterangan
Artikel jurnal ke-1.	
Nama jurnal yang dituju	*Bioscience Research*
Klasifikasi jurnal	Internasional
Impact factor jurnal	0.4500
Judul artikel	Growth responses of tobacco (Nicotiana tabacum L.)varieties to water logging stress
Status naskah	Sudah terbit
Artikel jurnal ke-2.	
Nama jurnal yang dituju	Data in Brief
Klasifikasi jurnal	Internasional
Impact factor jurnal	1.4500
Judul artikel	Data of root anatomical responses to periodic waterlogging stress of tobacco (Nicotiana tabacum) varieties
Status naskah	Sudah terbit

Artikel jurnal ke-3.	
Nama jurnal yang dituju	Journal of Physics
Klasifikasi jurnal	Internasional
Impact factor jurnal	1.4500
Judul artikel	Particular variety of tobacco Nicotiana tabacum) exhibits distinct morphological and physiological responses against periodic waterlogging stress
Status naskah	Sudah terbit

2. BUKU AJAR

	Keterangan

3. PEMBICARA PADA PERTEMUAN ILMIAH (SEMINAR/SIMPOSIUM)

	Keterangan

4. SEBAGAI INVITED SPEAKER

	Keterangan
Judul makalah	Potential of Tobacco Plants (Nicotiana tabacum) Tolerant of Waterlogging as Bioinsecticide Material
Penulis	Pertama
Penyelenggara	Departemen Of Biology Institut Teknologi Sepuluh Nopember
Waktu Pelaksanaan	10/13/2018 12:00:00 AM
Tempat Pelaksanaan	Hotel Harris Surabaya
Skala Pertemuan	Internasional
Status naskah	Sudah dilaksanakan
Judul makalah	Profile of Protein Levels Some Tobacco Varieties (Nicotiana tabacum L.) on Waterlogging Stress
Penulis	Ketiga
Penyelenggara	FACULTYOFSCIENCEANDTECHNOLOGYMAULANA MALIK IBRAHIM STATE ISLAMIC UNIVERSITYOF MALAN

Teknologi Tepat Guna	Teknologi Tepat Guna ini berupa Produk Biopestisida yang ramah lingkungan dengan bahan dasar daun tembakau yang mendapat cekaman genangan secara periodik

, 21 - 11 - 2018
Ketua,

(Drs HERY PURNOBASUKI M.Si, Ph.D)

Waktu Pelaksanaan	10/7/2017 12:00:00 AM
Tempat Pelaksanaan	' FACULTYOFSCIENCEANDTECHNOLOGYMAULANA MALIK IBRAHIM STATE ISLAMIC UNIVERSITYOF MALAN
Skala Pertemuan	Internasional
Status naskah	Sudah dilaksanakan
Judul makalah	Response morphology and anatomy of tobacco (Nicotiana tabacum L.) plant on waterlogging
Penulis	Ketiga
Penyelenggara	Universitas Brawijaya
Waktu Pelaksanaan	7/20/2017 12:00:00 AM
Tempat Pelaksanaan	Universitas Brawijaya
Skala Pertemuan	Internasional
Status naskah	Sudah dilaksanakan
Judul makalah	Respon of Nicotiana tabacum plant to Waterlogging Stress DuringVegetatif Stage
Penulis	Ketiga
Penyelenggara	SOCIETY FOR INDONESIAN BIODIVERSITY & GAJAH MADA UNIVERSITY
Waktu Pelaksanaan	3/18/2017 12:00:00 AM
Tempat Pelaksanaan	Hotel UNIVERSITY CLUB UNIVERSITAS GAJAH MADA Jalan Pancasila no.2 Bulaksumur, Univeritas Gajah Mada, Yogyakarta
Skala Pertemuan	Internasional
Status naskah	Sudah dilaksanakan

5. UNDANGAN SEBAGAI VISITING SCIENTIST PADA PERGURUAN TINGGI LAIN

	Keterangan

6. CAPAIAN LUARAN LAINNYA

Capaian	Uraian

PUBLIKASI ILMIAH

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RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2017 14(3): 574-581. OPEN ACCESS

Growth responses of tobacco (*Nicotiana tabacum* L.) varieties to water logging stress

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Having high economic value makes tobacco becomes favorite industrial crop cultivated in Indonesia. However, several negative environmental conditions, including heavy rainfall season, affect the successfulness of tobacco cultivation. Heavy rainfall season is the major cause of waterlogging stress, which affect plant productivity. This study aims to determine the growth responses of some tobacco varieties under *waterlogging stress* and to evaluate its tolerance against *waterlogging stress*. Five tobacco varieties were used in this study, including var. Bligon 1, Bojonegoro 1, Grompol Jatim 1, Kemloko 1 and Prancak 95. The results were analyzed using two way Anova test and followed by Tukey test. The results showed that *waterlogging stress* decreased plant growth, induced the formation of aerenchyma tissues and adventitious root. In addition, this environmental stress also induced stomatal closure. In this present study, we observed that var. Bligo 1 and Grompol Jatim 1 are medium tolerant against waterlogging stress. Meanwhile, var. Bojonegoro 1 is considered as tolerant variety. Furthermore, the rest of tobacco varieties are sensitive to *waterlogging stress*. The overall results demonstrated *waterlogging stress* affect plant growth; induce stomatal closure, adventitious root and aerenchyma root formation. Moreover, each tobacco variety showed different level of tolerance against waterlogging stress

Keywords: *Nicotiana tabacum*, Plant growth, Sensitivity Index, Tolerant, Varieties, Water logging Stress

INTRODUCTION

Tobacco is considered as industrial crops possessing high economic value (Simpson and Ogorzaly, 2001). Not only being used as the main material in cigarette industry, tobacco has been used in medicinal and agricultural-based products, including pesticides (Simpson and Ogorzaly, 2014). However, the cultivation of this promising plant face major environmental constraint, including high rainfall season, which is typically occurred in tropical region. This uncertain season might cause flooding or waterlogging stress.

One of the major effects of waterlogging stress is the emergence of hypoxic or anoxic condition in the plant root system (Smith et al., 2010). In addition, the waterlogging stress causes increased soil density, plant phytotoxicity due to the formation of toxic compounds, decreased in soil pH and oxygen supply (Parent et al., 2008). Previous research has reported that decreased in soil pH causes plant cell damage and often leads to acceleration of cell death in sensitive to waterlogging stress plants (Hodson and Bryant, 2012).

However, tolerant plants may survive under waterlogging stress by developing adaptation

mechanisms, which includes anatomical and morphological adaptation as well as metabolic regulation mechanisms (Susilawati et al., 2012). An example of anatomical adaptation is the formation of aerechyma tissues in the root system. In maize, hypoxic condition stimulates the ethylene biosynthesis. This may induces subsequently to cell death in the root cortex, giving rise further to the formation of aerenchyma at the root (Rajhi et al., 2010).

In addition, plants also develop adventitious root when they are suffering from the waterlogging stress. The presence of adventitious root might help plants to easily absorb the oxygen (Dawood et al., 2014). Research on tomatoes (*Solanum lycopersicum*) indicates that one week treatment of waterlogging stress increases the formation of adventitious roots (Vidoz et al., 2010). This study aims to determine the growth responses of some tobacco varieties (var. Bligon 1, Bojonegoro 1, Grompol Jatim 1, Kemloko 1 and Prancak 95) under waterlogging stress and evaluate its tolerance againts waterlogging stress.

MATERIALS AND METHODS

Tobacco varieties used in this study include var. Bligon 1, Bojonegoro 1, Grompol Jatim 1, Kemloko 1 and Prancak 95, which were obtained from the Indonesian Sweetener and Fiber Crops Research Institute (ISFRI). The study was conducted at the green house of the Laboratory of Plant Biosciences and Technology, Department of Biology, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia.

Preparation of growing media

:Growing media used in this study is composed of mixture of soil and compost with a ratio of 1: 1. The total weight of the planting medium was 1 kg, where 1 g additional NPK fertilizer was added to each polybags.

Preparation and Planting of Tobacco Seedlings:

Tobacco seeds were germinated in potting trays containing growing media (soil and compost mixture in the ratio 1: 1). Germination is done for 4 weeks. Furthermore, young juvenile tobacco plants are transferred to polybags that have contained planting media (Hurng et al., 1994).

Waterlogging stress treatment:

Tobacco plants aged 40 days after planting (dap) were treated with waterlogging stress (Hurng et al., 1994).Each varieties were subjected to waterlogging stresses of 150%, 175%, and 200% of the field capacity. Meanwhile, the control plants were treated with 100% of the field capacity. Waterlogging stress were conducted for 10 days. The volume of water in each treatment is regularly adjusted and sustained for 10 days of treatment.

Plant growth parameters used in this study include plant height, number of adventitious roots, canopy-root ratio, the total number of aerenchyma formation. The sensitivity index was measured against the total dryness observation parameter by the equation:

$$S = \frac{\left(1 - \frac{Yp}{Y}\right)}{\left(1 - \frac{Xp}{X}\right)}$$

Information:

S = Sensitivity Index of waterlogging stress
Yp = the average values of a treated variety
Y = the average values of a non-treated variety
Xp = the average of total treated varieties
X = the average of total non-treated varieties

The criteria for tolerance level of *waterlogging stress* are: **tolerant variety** (when S < 0,5), **medium tolerant variety** (0.5 ≤ S ≤ 1.0), and **sensitive variety** (S > 1.0).

The data obtained were analyzed using ANOVA Two Way and followed by Tukey test. Aerenchima tissue observation was analyzed descriptively.

RESULTS

Tobacco Plant Morphology Response At Waterlogging Stress

Our statistical analysis (Table 1) showed that waterlogging stress significantly influences the plant height, canopy-root ratio, root length and adventitious root quantity. Based on Table 1 and Figure 1, plant height growth tends to decrease when the plants were treated under waterlogging condition. Maximum treatment at 150% of field capacity, the crops showed a significant decrease in growth (plant height), and further decrease with increasing waterlogging stress. Similarly, the root length begins to decrease significantly at 150% of field capacity (Figure 2). Decrease in plant growth further affects the root and canopy ratios. Based on Figure 3, it is known that the ratio of root canopy increases following an increment of field capacity (waterlogging stress). Finally, plants adapt by inducing and accelerating the formation of adventitious roots (Figure 4).

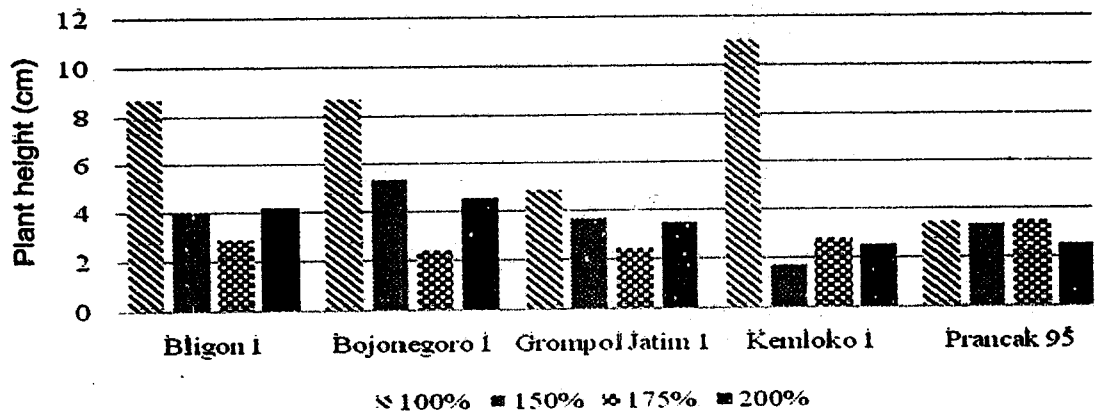

Figure 1. Plant height response of tobacco varieties after waterlogging stress treatment

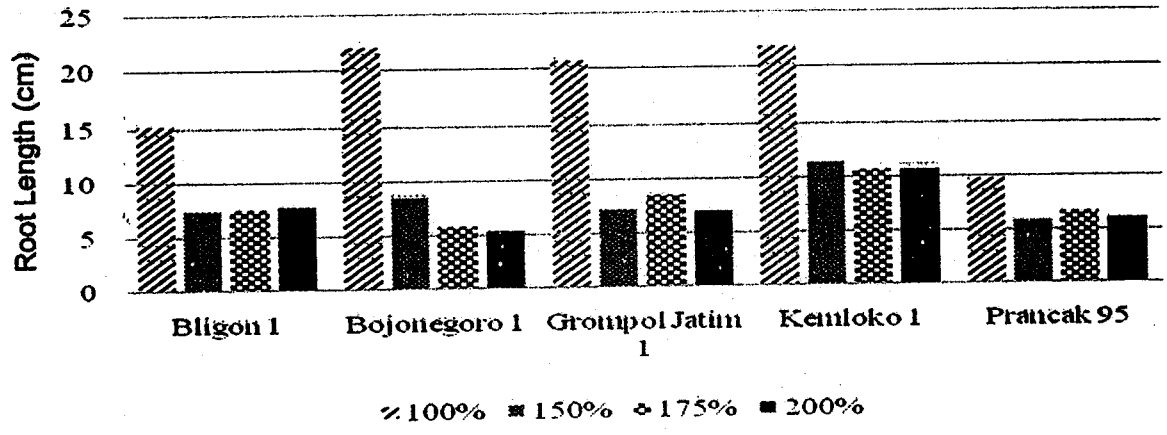

Figure 2. Growth Response in root length of tobacco varieties after *waterlogging stress* treatment

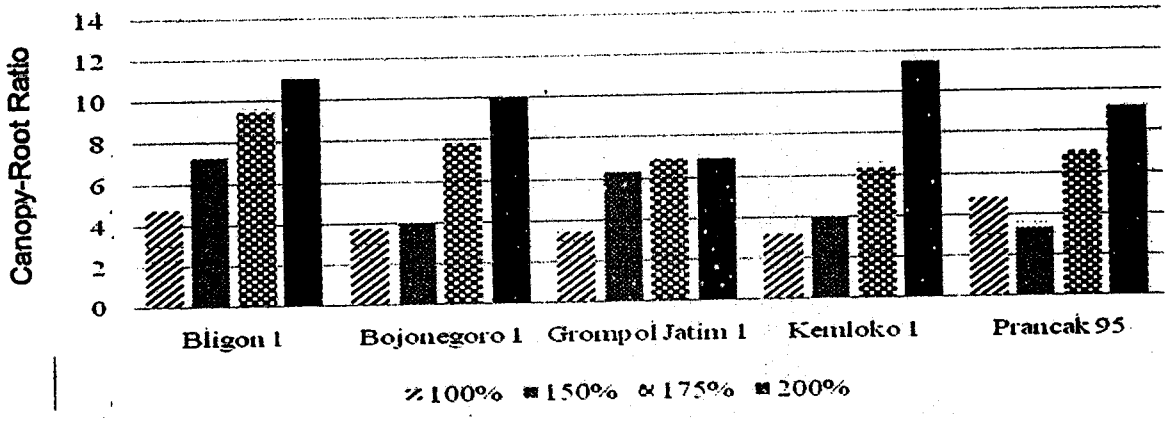

Figure 3. Growth Response in canopy-root ratio of tobacco varieties after waterlogging stress treatment

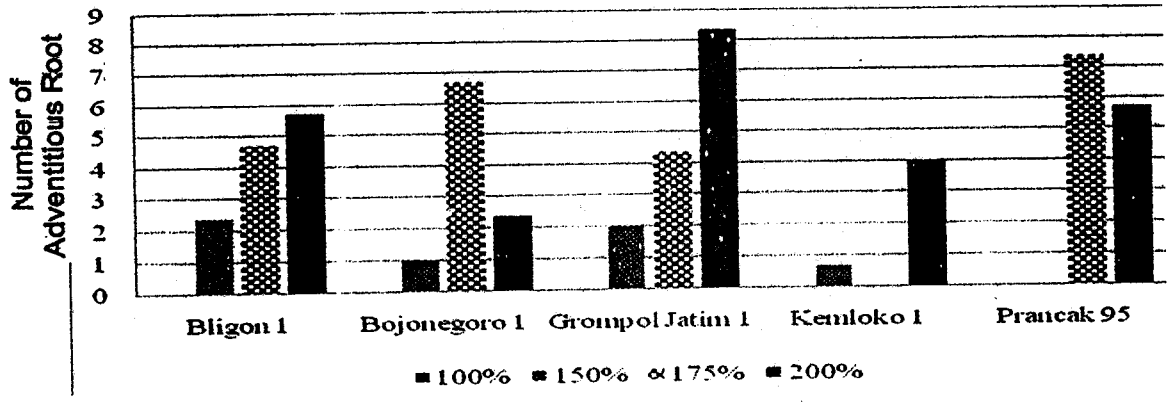

Figure 4. Number of adventitious roots of tobacco varieties after waterlogging stress treatment.

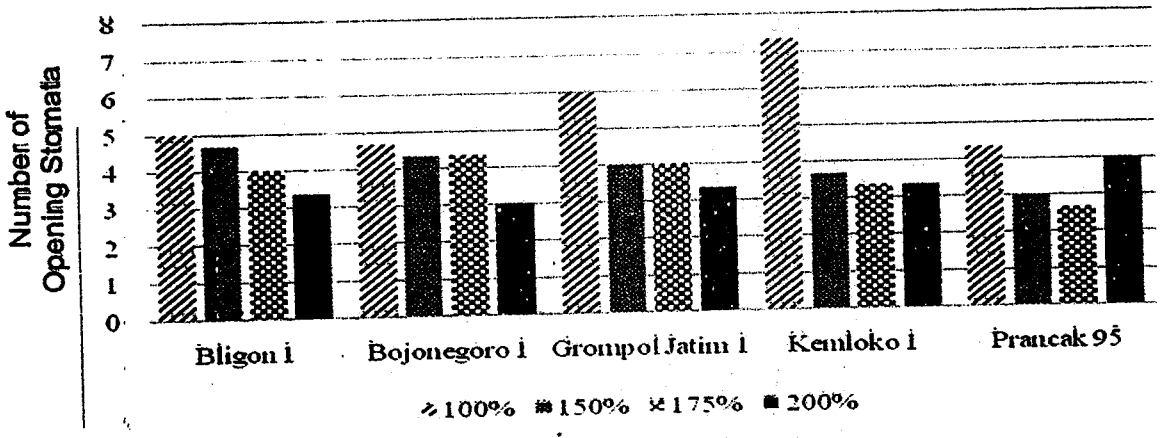

Figure 5. Number of opening stomata in tobacco varieties after waterlogging stress treatment.

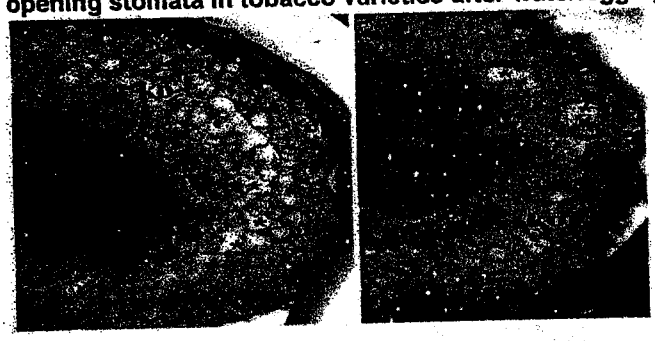

Figure 6. Aerenchymal observation of tobacco Bojonegoro 1 varieties treated with *waterlogging stress*: (A) Plant tobacco control; (B) Plant tobacco Treatment (ep: epiderm; co: cortex; en: endoderm; ste: stele; asterisk (*) aerenchy

Tobacco Plant Anatomy Response At Waterlogging Stress

Anatomical observation in response to waterlogging stress includes morphological observation of the stomata and plant aerenchyma tissue observation. Our data (Table 1) showed that waterlogging stress had significant effect to total number of open stomata. Based on Table 1 and Figure 5, it is known that the total number of opening stomata decreases with an increase of waterlogging stress level. Root anatomical observation showed the formation of aerenchyma (Figure 6).

Table 1. ANOVA for Plant Height, Root Length, Canopy/Root Ratio, Number of Adventitious Roots and Number of Stomata Using Adjusted SS for Tests

Source of variations	Tukey Test				
	Plant height (cm)	Root length (cm)	Canopy / Root ratio	Number of Adventitious Roots	Number of Opening Stomata
Varieties	0,308	0,000*	0,108	0,095	0,211
Waterlogging stress	0,000*	0,000*	0,000*	0,000*	0,000*
Varieties* Waterlogging stress	0,241	0,092	0,300	0,302	0,074

Note: The symbol "*" indicates high significant difference

Table 2. Sensitivity Index of Tobacco Varieties in Waterlogging Stress

Varieties	150%			175%			200%		
	S	MT	T	S	MT	T	S	MT	T
Bligon 1	√			√				√	
Bojonegoro 1			√			√			√
Grompol Jatim 1		√			√			√	
Kemloko 1		√		√			√		
Prancak 95	√			√			√		

Description: S = sensitive, MT = medium tolerant, T = tolerant

Sensitivity Index Tobacco Plants In Waterlogging Stress

Determination of the sensitivity index is based on the plant dry weight parameters. The tobacco plant sensitivity index at 150%, 175% and 200% stresses is presented in Table 2. Varieties Bojonegoro 1 seems to be more tolerant compared to others. Meanwhile var. Bligon 1 and Grompol Jatim 1 is considered as medium tolerant

DISCUSSION

Plants develop unique strategy to face adverse environmental condition. The later includes abiotic stress which seems to be more pronoounced in tropical region. Drought and flooding stress are two major causes of low yield of industrial crops. To better adapt and response this environmental condition, plants perform a complex combination of anatomical, physiological and molecular responses (Kumutha et al., 2009, Jadid et al., 2017). In addition, excessive production of reactive oxygen species (ROS) during the environmental stress could also severely affect plant growth. Therefore, environmental stress could also induce the formation of antioxidant compounds. The later is not only useful for human health (Jadid et al., 2016) but also vital for plant growth and development, especially when plants are subjected to severe environmental stress. Additionally, some reports also demonstrate that abiotic stress could enhance chlorophyll breakdown, protein degradation, decrease in membrane permeability and stability (Jadid et al., 2017), slower leaf expansion, stomatal closure and petiol epinasty (Lin et al., 2006). Based on Figure 1 - 2, the plant height and root length of tobacco varieties tend to decrease following the level of waterlogging treatment. At the 150% of field capacity level, the plants begin to decrease its plant growth and it was subsequently decline following the increase of waterlogging stress levels. This might be caused by the nutrient and water competition within the vegetative organs such as roots, leaves and stems occured during the treatment. This competition could inhibit the plant height but in contrast, it could also increase the leaf area of tobacco varieties.Lin et al., (2006) reported also that waterlogging stress caused

hypoxic to anoxia conditions. This might slow the cellular respiration within the root tissues. Thereby, plants tend to temporary change cellular respiration pathway from aerobic to anaerobic respiration / fermentation. However, Fermentation is less efficient in converting ADP to ATP, compared to aerobic respiration. Limited availability of metabolic energy would inhibit multiple biological processes in the plant including cell division, water and nutrient uptake and other metabolic processes.

The root-canopy ratio is the ratio between the canopy's dry weight and root dry weight. According to Firdaus et al. (2013), high canopy-root ratio indicates good upper vegetative growth. In this study, we observed an increase of canopy-root ratio of treated varieties following an increase of waterlogging stress levels (Figure 3). This suggests that root growth is more inhibited than shoot growth during waterlogging stress. In addition, this also suggests that there were unbalanced accumulation of photosynthate products between shoot and root. The canopy-root ratio begins to increase significantly at 175% of waterlogging stress. This result is in line with Florentine and Fox (2002) which states that in Eucalyptus victrix, E. terminalis, and E. leucophloia, the ratio of canopy-root in the plant is higher than the unheated control plants

.Waterlogging stress further suppresses root growth compared to shoot growth (Lin et al., 2006). The change of cellular respiration pathway from aerobic to anaerobic might play role in this process, which subsequently result in cell damage and often leads to cell death in sensitive plants (Hodson and Bryant, 2012). The cell death phenomenon begins probably at root tissue since it is the first plant organ affected by waterlogging stress.

Adventitious roots were observably found in tobacco varieties treated with waterlogging stress.

This type of root is actually formed from organ other than root tissues. Adventitious roots arised after plants were treated with waterlogging stress (Figure 4). This formation is common plant adaptation against waterlogging stress. Based on the reference (Cronk and Fennessy, 2001), within a few days of flooded condition, some plants will initiate the formation of adventitious roots that grow laterally from the main stem. Adventitious root formation occurs due to the interaction of several plant hormones, auxin and ethylene (Akhtar and Nazir, 2013). In the absence of oxygen, the Krebs cycle cannot be run due to lack of a terminal electron acceptor for the oxidation of NADH. The next ATP can only be produced by fermentation, in which the first pyruvate is converted into lactate. But this has not happened in a long time, as well as a decrease in pH in the cytosol causes inhibition of lactate dehydrogenase activity and turned into ethanol fermentation. Under anaerobic conditions, the proton released from the vacuole to the cytoplasm will increase the acidity caused by lactate dehydrogenase

.Deoreased pH and occurrence of cytosolic acidosis are the main causes of cell damage, and often lead to cell death in sensitive plants (Hodson and Bryant, 2012). This further causes root damage and inhibition of auxin transport to the root. Based on reference (Cronk and Fennessy, 2001), auxin is involved in the formation of adventitious root. Diffusion of auxin to the root of was inhibited in less-oxygen root. This might cause the accumulation of auxin in a zone between the shoot and root in which adventitious roots are formed. Adventitious roots help the absorption of water and nutrients in plants that are tolerant to waterlogging stress. Adventitious roots also facilitate the diffusion of end-products alcohol fermentation within the plant to avoid alcohol accumulation.

Stomata consist of a pair of guard cells and some additional cells. Stomata serve to regulate the assimilation of carbon dioxide (CO_2) and the transpiration process through changes in guard cell turgidity. This specific feature is critical for the global water-carbon cycle and the ability of plants to respond to environmental changes (Gan et al., 2010). Based on Table 1 and Figure 5, waterlogging causes decrease of opening stomata. This indicates that there has been a root damage that causes an inhibition of water absorption. The plants further respond with stomatal closure to reduce the transpiration rate. At physiological level, stomatal closure reduces transpiration but inhibits photosynthetic process. This response can occur within a few hours or days, depending on the degree of tolerance of each plant to the waterlogging (Striker, 2012). Excessive ground water inhibits respiration of roots and water and nutrient absorption from soil, induces stomatal closure and finally dramatically.

Reduces plant growth.

Determination of the sensitivity index is based on the total dryness (total dry weight) observation, since the dry weight indicates the positive or negative effect of plant growth. Based on the observation, the most tolerant plants tolerant to the waterlogging stress (highest level of waterlogging stress, 200%) is var. Bojonegoro 1, while Bligon 1 and Grompol Jatim 1 is considered as medium tolerant (Table 2). These results might reflect their native habitat where these three varieties are commonly cultivated in the area with an excess of water. Var. Bligon 1, Bojonegoro 1, and Grompol Jatim 1 are more suitable for being cultivated in ex paddy fields. Var. Prancak 95 is sensitive to all level of waterlogging stress. This is in line with the native condition where var. Prancak 95 is cultivated. This variety is commonly grown in dry climate area. Based on the reference (Anonymous, 2012), varieties Prancak 95 are selected from local varieties derived from Prancak, District Pasongsongan, Sumenep, Indonesia. This variety is more suitable to be grown in dry land.

CONCLUSION

The overall results demonstrated waterlogging stress affect plant growth, induce stomatal closure, adventitious root and aerenchyma root formation. In addition, each tobacco variety showed different level of tolerance against waterlogging stress. Bojonegoro variety is a tolerant varieties in 200% of waterlogging Stress level.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

TN and NJ designed the experiments and analyze the data. HC performed the experiments. TN, HP, SH and NJ supervised all the biological experiments. TN and NJ wrote the manuscript. All authors read and approved the final version

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Particular variety of tobacco Nicotiana tabacum) exhibits distinct morphological and physiological responses against periodic waterlogging stress

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Abstract. Waterlogging Stress influences crop productivity, especially high commercial crop like tobacco, which is very sensitive to an excess of water. This type of environmental stress might happen because of unpredictable season occurred in tropical region, including Indonesia. Global climate change as a consequence of rapid growing industries all over the world might responsible for this. This study aims to investigate the response of some *Nicotiana tabacum* varieties which are treated with periodic waterlogging stress. Some morphological aspects including plant height, stem diameter, leaves width and the emergence of adventitious roots were investigated. In addition, total chlorophyll content was also measured as physiological parameter. Three different varieties are used such as var. Jepon Pelakean, Jinten, Manilo and Morakot. Data were analyzed using One-Way Annova followed by Tukey *post-hoc* analysis. In all morphological aspects var. Jepon Pelakean demonstrated best responses compared to other varieties. Meanwhile, we observed a sharp decline of total chlorophyll content in var. Manilo under this periodic waterlogging stress. The overall results suggest that each variety of tobacco possess a specific mechanism against periodic waterlogging stress.

1. Introduction

Tobacco plant is one of important trading commodity in the world, including Indonesia and therefore play a significant role in the national economy [1]. Global climate change, especially on agriculture sector in Indonesia has a significant effect on tobacco plant productivity. Tobacco productivity has been reported to decline due to biological damage caused by waterlogging [2]. This is particularly problematic since the frecuency of flood disaster has increased in the last [3]. The term of waterlogging or flood often used to describe the different situations in which excess of water can range from saturated soil water conditions (waterlogging) until the formation of the water column causing a thorough immersion in plants (complete submergence) [4]. Saturated soil results in in a drastic reduction of gas exchange rate [5]. This condition might provoke serial perturbation on the biological system of the plant, since oxygen is required to maintain aerobic respiration in submerged tissue [6]. Therefore, the survival of plants under these conditions depends on physiological, morphological and metabolic adaptations [7].

Morphological responses might obviously occurred when plant face the waterlogging stress. This includes the formation of adventitious roots and an increase in plant height. These will Additionally, plant also develops aerenchyma tissues for facilitating the oxygen distribution throughout the plant body [9]. An excess of water that drowns the aerial part of the plant would reduce external carbondioxide levels. The later will cause further decline of photosynthetic rate [10]. This condition is exacerbated by the changes in stomatal conductance [4] and a decrease in chlorophyll content [11]. This study will investigate the morphological and physiological responses of some tobacco varieties against periodic waterlogging stress.consequently result in an increased of aerial organs biomass [8].

2. Experimental Details

Seeding is done in mixed media tray containing vermicompos and coco peat (1: 1). Furthermore, the growth medium was added NPK (5 g / 1 medium) and Ridomil solution (0.5 grams / liter of water). Pricking was done using seedlings with 3-4 leaves. Plants that have reached the age of 60-70 das (possessing 4-5 leaves), were then placed into plastic container (40 cm x 30 cm x 20 cm). Each container is filled with 3 polybags of tobacco from the same variety. Stress treatment was firstly done by filling the container with water until reached 13 cm. This condition made the root of the tobacco plants saturated with water (soil waterlogging). This step was maintened for 5 days. The water level was continually maintained at 13 cm height (figure 1). Second step of treatment was done by adding water into the container until part of the stems and the first-second leaves drowned (partial submergence). This second step as also carried out for 5 days, and therefore, the total treatment was maintained. The morphological data includes plant height, stem diameter, leaf width and the number of adventitious roots. Physiological data collection includes the total chlorophyll content. Briefly, leaves were extracted using acetone. The extracts were then analyzed using a spectrophotometer at wavelengths 663 and 645 nm. The experiment was designed using Completely Randomized Design (RAL) using 4 varieties namely Jepon Pelakean, Jinten, Manilo dan Marakot. All data was replicated three times. Data were analyzed using ANNOVA One Way followed by Tukey *post-hoc* test.

Figure 1. Waterlogging Stress treatment at First Stage

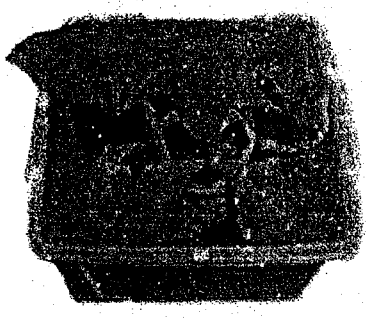

Figure 2. Waterlogging Stress treatment at Second Phase (*Partial submergence*)

3. Results and Discussion

Morphological responses generally carried out by the plant, including the formation of adventitious roots and an increase in plant height. This resulted in increased biomass aerial organs, namely the trunk organs. The morphological response in plants to facilitate the distribution of oxygen to the tissues were soaked through aerenchyma network. The observed morphological parameters plant height, stem diameter, leaf width and the formation of adventitious roots are presented in Table 1.

Table 1. Morphological Characteristic of Tobacco Plant Varieties In Condition of Periodic Waterlogging Stress

Morphological Character	Tobacco Plant varieties							
	Jepon Palakean		Jinten		Manillo		Marakot	
	1	2	1	2	1	2	1	2
Plant Height (Cm)	9.63	10.5	11.53	10.5	14.2	10.27	14	9.03
Stem Of Diameter (Cm2)	8.23	9.13	6.77	8.87	8.43	8.03	7.83	8.77
Leaf Widht (Cm2)	11.07	12.1	8.47	10.5	12.23	9.93	11.8	11.1
Number of Adventitious Roots	0	11.33	0	5.67	0	3	0	10.33

Description: (1) Control Plants and (2) Treatment Plants of Periodic Waterlogging Stress

Plant height is a common parameter that should be measured in response to waterlogging stress. This can occur as a result of the interaction of plant hormones, such as abscisic acid (ABA), Gibberellins (GA) and Ethylene (Jackson, 2008). Based on our observation, var. Jepon Pelakean showed an increased of plant height compared to other varieties (Table 1). Meanwhile, three other test varieties showed a decreased of plant height compared to control. Basically, each variety has a different response based on internal interactions within the plant hormone. This is in accordance with previous study, conducted by [15], which showed that under waterlogging stress, two different rice varieties also demonstrated distingush responses. This might be due to hormonal factors involved, such as ethylene, ABA and GA. Var. Jepon Pelakean exhibited common morphological response under waterlogging stress, ie stem hypertrophy. This type of adaptive response is characterized by the production of GA that in some extent it depends on the availability of ABA. Basically, the accumulation of ethylene is also increased under hypoxic conditions. This will lead to a decrease of ABA accumulation through the inhibition of the expression of 9-cis-epoxycarotenoid dioxygenase and via activation of ABA into phaseic acid (PA). This certainly causes a decrease of endogenous ABA that is also required to enhance the expression of GA3 oxidase (an enzyme which catalyzes the conversion of PA into an active gibberellins / GA1). In addition, an increase of ethylene will also result in a lower pH in the apoplast which helps to stimulate the cell wall loosening, a critical step in cell elongation. Stem hypertrophy as a consequence of either hypoxic or anoxic conditions, possibly occurred because of the development of white spongy tissues. This type of tissue is further called as aerenchyma, which is formed externally from felogen and is a homologue of cork tissue. The aerenchyma tissue functions to increase the gas movement between submerged and non-submerged tissues. Some reports have demonstrated the formation of aerenchyma tissues as well as stem hypertrophy in some species such as *Lythrum salicaria*, *Lotus uliginosus*, *L. Tenuis*, *Glycine max*. In contrast to the result obtained in the var. Jepon Pelakean, the other varieties, var.Morakot, Manilo and jinten showed the opposite responses. These varieties showed reduced plant height, compared to control. Dubois et al. (2011) has demonstrated that lowland-rice varieties showed different responses compared to deepwater-rice varieties that undergo stem hypertrophy. Lowland-rice varieties exhibited an increased of gene expression that functions to suppress the formation of GA SUB1A through an increased of expression of SLR1 and SLRL1 repressor. Thus, it can be assumed that var. Marakot, Manilo, and Jinten might exhibit an overexpression of the SUB 1A-like gene that plays a role in GA biosynthesis. Then SUB 1A-like gene would regulate the SLR1 and SLRL1 which suppresses the synthesis of GA. The later would end up with an inhibition of stem elongation.

An increased of stem diameter correlate with the formation of aerenchyma tissue when the plants are facing the waterlogging stress. The formation of this type of tissues might increase the stem diameter. Plant physiological response to waterlogging stress depend on the tolerance level of the plant species. These physiological responses can be stomatal closure, reduction in transpiration and photosynthesis inhibition. Plants also respond to this environmental stress by regulating protein synthesis specifically for anaerobic conditions (anaerobic polypeptide / ANPS). In addition, reduce chlorophyll content could be also considered as physiological response of plants under waterlogging stress [4]. Simultaneous decline of total chlorophyll content in all tested varieties was obviously observed in this study. The highest chlorophyll content can be observed in var.Morakot under this type of stress, which only decreased by 2.8 %, followed by var.Jepon Pelakean in which its chlorophyll has decreased by 7.6 % compared to controls. Meanwhile, biggest decline of chlorophyll content occurred in var.Manilo that is equal to 41.5 %, followed by var.Jinten (33.8 %), compared to controls. Thus, it can be suggested that var.Morakot and Jepon Pelakean possess better responses in term of its total chlorophyll content compared to others.

4. Conclusion

Tobacco var. Jepon Pelakean demonstrated best responses compared to other varieties in all morphological parameters. Meanwhile, we observed a sharp decline of total chlorophyll content in var. Manilo under this periodic waterlogging stress. The overall results suggest that each variety of tobacco possess a specific mechanism against periodic waterlogging stress.

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Data Article

Title: Data of Root anatomical responses to periodic waterlogging stress of tobacco (*Nicotiana tabacum*) varieties

Authors: Hery Purnobasuki[a*], Tutik Nurhidayati[b], Sucipto Hariyanto[a], Nurul Jadid[b]

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Abstract

The data of root anatomical structure and the formation of aerenchyma tissues of five varieties of tobacco under waterlogging stress were obtained by modified paraffin method. Each tobacco varieties performed distinct anatomical adaptation response, including changes of cortical tissue, stele diameter, xylem diameter and the formation of aerenchyma under periodic waterlogging stress.

Keywords : *Nicotiana tabacum, Periodic Waterlogging Stress, Anatomy of the roots*

Specifications Table

Subject area	*Biology*
More specific subject area	*Anatomy plant biology*
Type of data	*Figures and text*
How data was acquired	*Periodic waterlogging Method, paraffin method, data and image analysis*
Data format	*Analyzed*
Experimental factors	*Five tobacco varieties were traited under periodic waterlogging stress for 14 days, inlcuding 7 days with waterlogging conditions and followed by 7 days treatment of flooding conditions.*
Experimental features	*Tobacco varieties used in this study include var. Jepon Palakean, Srumpung, Morakot, Somporis and Manilo. The observation of root anatomy was conducted using modified paraffin method.*
Data source location	*Department of Biology, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia*
Data accessibility	*The data are available with this article*

Value of the Data

- The tobacco plant performs anatomical adaptation response of the roots under periodic waterlogging stress conditions through changes in cortical tissue, stele diameter, xylem diameter and the formation of aerenchyma.
- Data on root anatomical responses might be useful for further study on tobacco plant breeding.
- Data provided in this article could be combined with physiological and molecular study to elucidate the tobacco response mechanism against waterlogging and flooding stress

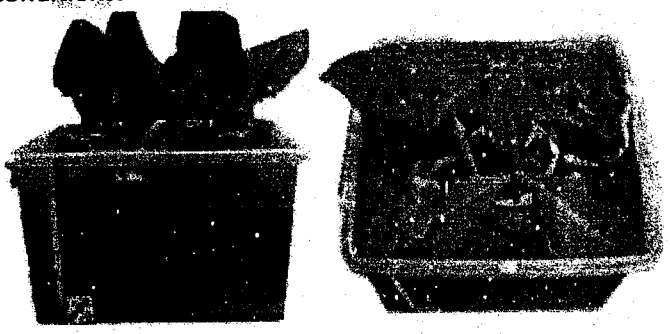
1. Data

The data on Fig. 1 showed the waterlogging stress treatment and Fig. 2 showed the cross-section of fifth variety of tobacco root under periodic waterlogging stress. Our data clearly showed the anatomical differences between treated plant and control. All of treated plants have bigger size of all root paramater and much number in aerenchyma, epidermal and endodermal cells. All varieties showed the formation of aerenchyma tissue after being treated with waterlogging and flooding stress (Figs 2-3). During treatment, tobacco varieties exhibited different root anatomical responses. Tobacco var. Jepon Palakean, Marakot and Manilo showed an increase of cortex thickness (more than 60% in waterlogging and more than 100% in flooding), diameter of stele and xylem (more than 75% in waterlogging and more than 40% in flooding). In contrast, var. Srumpung and Somporis exhibited a decrease of cortex thickness, diameter of stele and xylem (Table 1).

2. Experimental Design, Materials, and Methods

2.1 Periodic waterlogging stress treatment

Tobacco seedlings aged 65 DAS (days after sowing) were grown under the periodic waterlogging condition in a plastic container measuring 40 cm x 30 cm x 20 cm (Fig 1). Five tobacco varieties were used in this study including var. Jepon Palakean, Srumpung, Marakot, Somporis and Manilo. Periodic waterlogging stress treatment with a total 14 days was divided into 7 days in waterlogging conditions and 7 days under flooding conditions.



(A) (B)

Figure 1. Treatment of periodic waterloging stress in Tobacco (*Nicotiana tabacum*): A.Waterlogging condition and B. Flooding condition

2.2.Sample Preservation Tobacco's Roots

Root samples were firstly washed before being used for further analysis. Samples were prepared as ± 2 cm size. Subsequently, samples were fixed in FAA solution (formalin: acetic acid: 95% alcohol = 50 ml: 50 ml: 900 ml for every 1 liter of solution) in a desiccator tool. Hydration process is then performed in a desiccator for 3x30 minutes. The FAA solution was then removed, and the sample was stored in a 70% alcohol solution.

2.3.Observation of root anatomical structure

The cross-sectional root anatomical structure was prepared using modified paraffin method. Procedures of the modified paraffin method used in this study were: (1) gradual dehydration with alcohol; (2) redehydration; (3) immersion through paraffin: dehydrant 1: 1;(4) Embedding;(5) Cutting using microtome;(6) Staining; (8) mounting using entelan. Root anatomical observation was conducted using light microscope with a camera Olympus CX 21 OPTILAB. Quantitative observations of root anatomical roots include total root diameter, stele diameter, epidermal thickness, cortical thickness, endodermic thickness, xylem thickness, xylem diameter, aerenchyme cell count and aerenchyme cell length. The data were analyzed by analysis of variance two way followed by Tukey test.

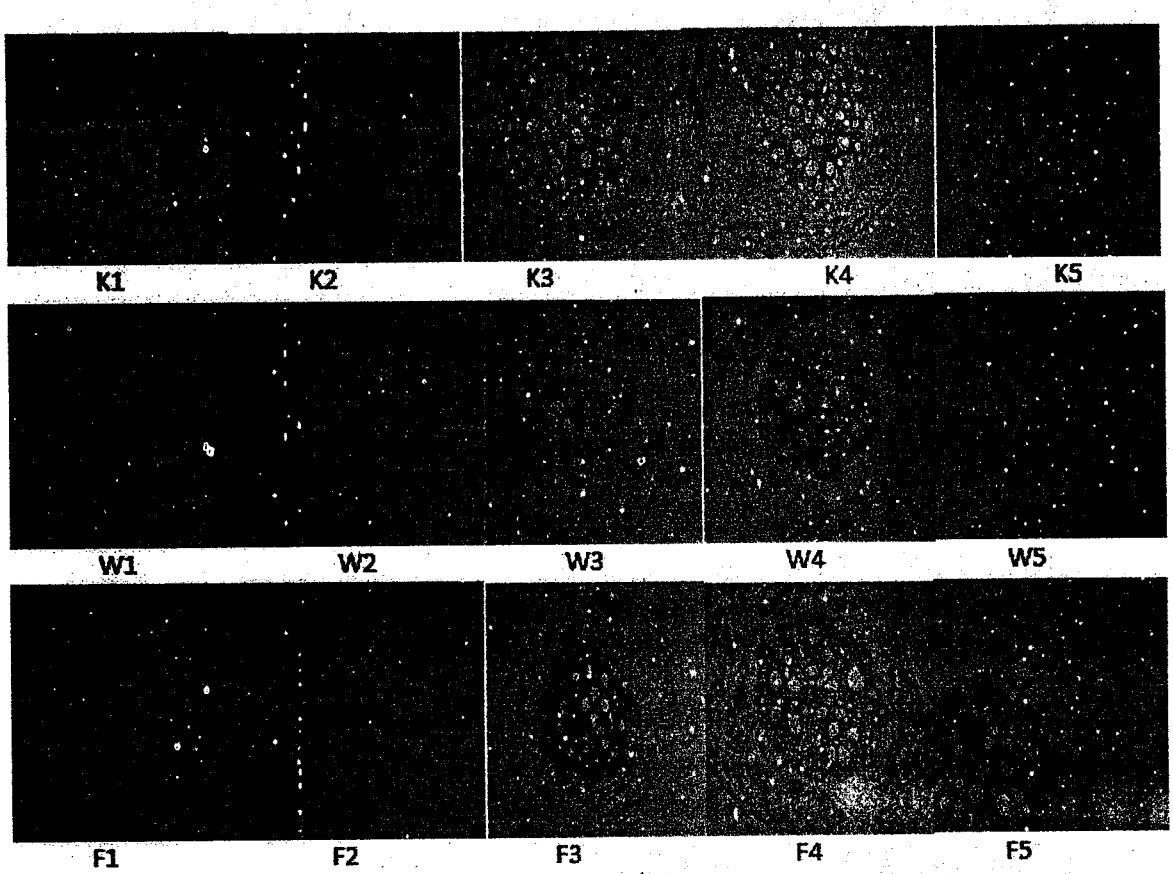


Figure 2. Cross-section of Fifth Root Varieties of Tobacco Plants under Periodic waterlogging (K: Tobacco Control Plants W: Tobacco Plants In *Waterlogging* Stress F: Tobacco Plants In *Flooding* Cash: 1. Var Jepon Palakean 2. Var Somporis3. Var, Marakot, 4. Var Srumpung, 5. Var Manilo).Observations were done using the Olympus CX 21 Light Microscope With Optilab Camera At Magnification (100X).

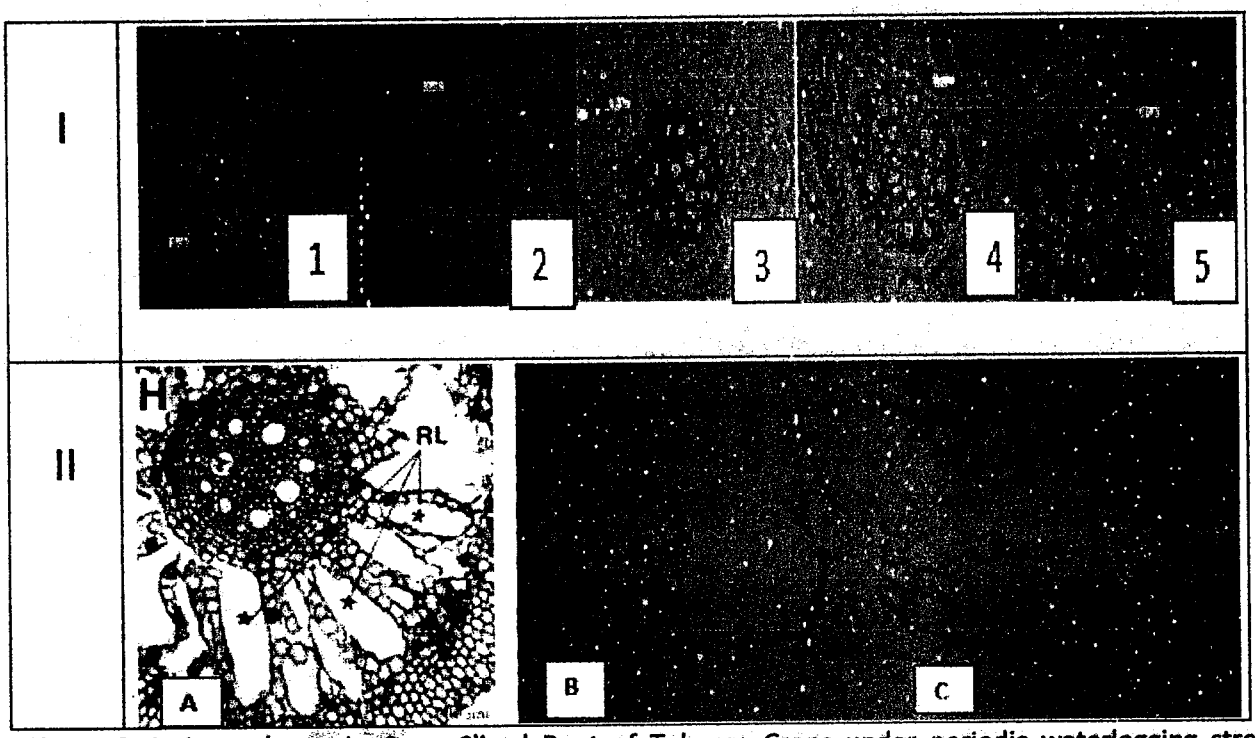


Figure 3. I: Aerenchyma In Cross Sliced Root of Tobacco Crops under periodic waterlogging stress Periodically / Flooding: (1. Var Jepon Palakean 2. Var Somporis; 3. Var. Marakot; 4. Var Srumpung; 5. Var.Marakot: Observations Using the Olympus CX 21 Light Microscope With Optilab Camera At 100X Magnification); II:II: A: *Typa latifolia* Root with radial type lisogeny Jung et al., 2008 was used as literature standard;Arrows indicate aerenchyme: (B: aerenchyme Var Somporis And C: Var.Marakot: Observation Using the Olympus CX 21 Light Microscope With Optilab Camera At 400X Magnification).

Table 1. Various Root Anatomical Characters of Tobacco Varieties under Periodic Waterlogging Stress

Parameter	Treatment	Tobacco Plant Varieties				
		Palakean	Somporis	Marakot	Srumpung	Manilo
Root Diameter(µm)	W0	1356,9±1,43ab	784,47±0,05ab	1618,37±0,36ab	1302,8±0,23ab	1638,77±0,27ab
	W1	1639,6±0,43ab	705,93±0,42ab	1585,53±0,54ab	4410±0,71ab	1274±0,88ab
	F0	721,67±0,30ab	1246,1±0,48ab	1281,87±0,45ab	1648,3±0,17ab	1627,37±0,42ab
	F1	1256,57±0,22ab	846,4±0,42ab	1470,33±0,26ab	1421,4±1,40ab	1670,13±0,07ab
Stele Diameter (µm)	W0	484,13±3,75ad	616,93±0,36ad	981,7±0,14ce	904,57±0,11be	715,57±0,91ce
	W1	833,33±0,17be	562,83±0,12ad	1016,57±0,44ce	658,8±0,21ad	892,67±1,14ad
	F0	348,13±0,19ad	721,67±0,22ad	672,8±0,13ad	842,07±0,46ae	733±0,37ad
	F1	760,07±0,13bd	433,73±0,49ad	751,33±0,68ad	800,2±0,60ad	1332,9±0,52ce
Epidermal thickness(µm)	W0	71,43±0,03bd	107,7±0,05bd	171,9±0,07bd	168,5±0,02bd	177,4±0,04bd
	W1	41,13±0,33ac	28,23±0,09ac	45,43±0,19ac	35,53±0,05ac	66,87±0,23bd
	F0	71,43±0,22bd	107,7±0,10bd	171,9±0,01bd	168,5±0,12bd	177,4±0,12bd
	F1	30,23±0,01ac	23,73±0,05ac	37,93±0,06 ac	32,13±0,09ac	31,23±0,07ac
Cortex thickness (µm)	W0	158,2±0,24ade	65,53±0,17ad	79,13±0,62ad	110,33±0,17ad	155,8±0,16ad
	W1	244,47±0,21bde	42,57±0,12ad	140,83±0,37ad	108,9±0,37ad	224,6±0,04bde
	F0	86,27±0,05ad	157,97±0,22ade	201,7±0,24bde	257,83±0,58bde	225,37±0,45bde
	F1	156,4±0,39ade	155,27±0,22ade	300,8±0,61ce	249,53±0,28bde	514,53±1,14ce
Endodermal thickness (µm)	W0	156,03±0,31bd	51,07±0,20ac	227,03±0,63bd	85,2±0,22acd	120,5±0,13ad
	W1	141,2±0,21ad	50,17±0,02ac	126,87±0,63ad	172,73±0,59bd	111,47±0,15acd
	F0	44,67±0,07ac	122,23±0,10ad	92,27±0,13acd	92,73±0,34acd	207,7±1,03bd
	F1	55,03±0,34ac	45,4±0,11ac	88,33±0,04acd	53,9±0,18ac	168,97±0,44bd
Xylemthickness (µm)	W0	20,3±0,02ab	30,8±0,07ab	28,8±0,03ab	28,73±0,05ab	28,17±0,03ab
	W1	29,7±0,06ab	25,23±0,05ab	36,5±0,08ac	29,87±0,05ab	30,47±0,04abc
	F0	41,9±0,04ac	35,33±0,01ac	31,1±0,03ab	30,37±0,05ab	21±0,06ab
	F1	35,37±0,06ac	29,57±0,01ab	36,9±0,12ac	29,23±0,01ab	34,67±0,02ac
Xylem Diameter (µm)	W0	99,97±0,03ad	116,8±0,01bd	108,87±0,02bc	135,27±0,02bd	87,97±0,18ac
	W1	121,17±0,01bd	97,4±0,02ac	148,1±0,01bd	102,33±0,07ad	116,2±0,17ad
	F0	92,77±0,02ac	102,37±0,01ad	90,87±0,01ac	102,27±0,01ad	102,37±0,01ad
	F1	119,53±0,05bd	76,97±0,03ac	93,6±0,01ac	98,27±0,02ac	87,03±0,02ac
The number of aerenchyma	W0	3±0,00ac	1±0,01ab	2±0,02ab	2±0,01ab	3±0,01ac
	W1	8±0,05ac	5±0,03ab	4±0,02ab	5±0,01ab	6±0,02ac

cells	F0	2±0,01ab	2±0,02ab	1±0,01ab	2±0,01ab	1±0,01ab
	F1	12±0,01ad	10±0,02ad	11±0,02ad	8±0,03ac	7±0,00ac
Aerenchym Cell Length (µm)	W0	34,55±0,06ac	41,4±0,07ad	33,67±0,05ac	45,62±0,06ac	48,97±0,03ac
	W1	115,16±0,04be	97,23±0,09bd	93,37±0,03bd	123,17±0,03be	80,93±0,10ad
	F0	75,2±0,02ad	45,32±0,01ac	42,12±0,03ac	82,45±0,10ad	67,82±0,02ad
	F1	137,76±0,23be	138,63±0,06be	166,1±0,13bf	160,1±0,45bf	168,17±0,34be

Description: 1. numbers followed by the same letters in the same row and column for the measured parameters do not significantly different by Tukey Test at 5%; 2. Treatment Code: W0: Control 1; W1: Waterlogging; F0: Control 2; F1: Flooding

Funding sources

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Data Article

Data of root anatomical responses to periodic waterlogging stress of tobacco (*Nicotiana tabacum*) varieties

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Keywords:
Nicotiana tabacum
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ABSTRACT

The data of root anatomical structure and the formation of aerenchyma tissues of five varieties of tobacco under waterlogging stress were obtained by modified paraffin method. Each tobacco varieties performed distinct anatomical adaptation response, including changes of cortical tissue, stele diameter, xylem diameter and the formation of aerenchyma under periodic waterlogging stress.

Specifications table

Subject area	*Biology*
More specific subject area	*Anatomy plant biology*
Type of data	*Figures and text*
How data was acquired	*Periodic waterlogging Method, paraffin method, data and image analysis*
Data format	*Analyzed*

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http://dx.doi.org/10.1016/j.dib.2018.09.046

Experimental factors	*Five tobacco varieties were traited under periodic waterlogging stress for 14 days, including 7 days with waterlogging conditions and followed by 7 days treatment of flooding conditions.*
Experimental features	*Tobacco varieties used in this study include var. Jepon Palakean, Srumpung, Morakot, Somporis and Manilo. The observation of root anatomy was conducted using modified paraffin method.*
Data source location	*Department of Biology, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia*
Data accessibility	*The data are available with this article*

Value of the data

- The tobacco plant performs anatomical adaptation response of the roots under periodic waterlogging stress conditions through changes in cortical tissue, stele diameter, xylem diameter and the formation of aerenchyma.
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- Data provided in this article could be combined with physiological and molecular study to elucidate the tobacco response mechanism against waterlogging and flooding stress.

1. Data

The data on Fig. 1 shows the waterlogging stress treatment and Fig. 2 shows the cross-section of fifth variety of tobacco root under periodic waterlogging stress. Our data clearly showed the anatomical differences between treated plant and control. All of treated plants have bigger size of all root parameter and much number in aerenchyma, epidermal and endodermal cells. All varieties showed the formation of aerenchyma tissue after being treated with waterlogging and flooding stress (Figs. 2 and 3). During treatment, tobacco varieties exhibited different root anatomical responses. Tobacco var. Jepon Palakean, Marakot and Manilo showed an increase of cortex thickness (more than 60% in waterlogging and more than 100% in flooding), diameter of stele and xylem (more than 75% in waterlogging and more than 40% in flooding). In contrast, var. Srumpung and Somporis exhibited a decrease of cortex thickness, diameter of stele and xylem (Table 1).

2. Experimental design, materials, and methods

2.1. Periodic waterlogging stress treatment

Tobacco seedlings aged 65 DAS (days after sowing) were grown under the periodic waterlogging condition in a plastic container measuring 40 cm × 30 cm × 20 cm (Fig. 1). Five tobacco varieties were used in this study including var. Jepon Palakean, Srumpung, Marakot, Somporis and Manilo.

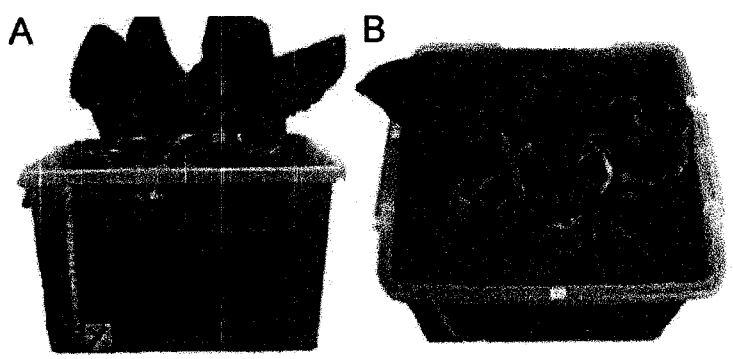
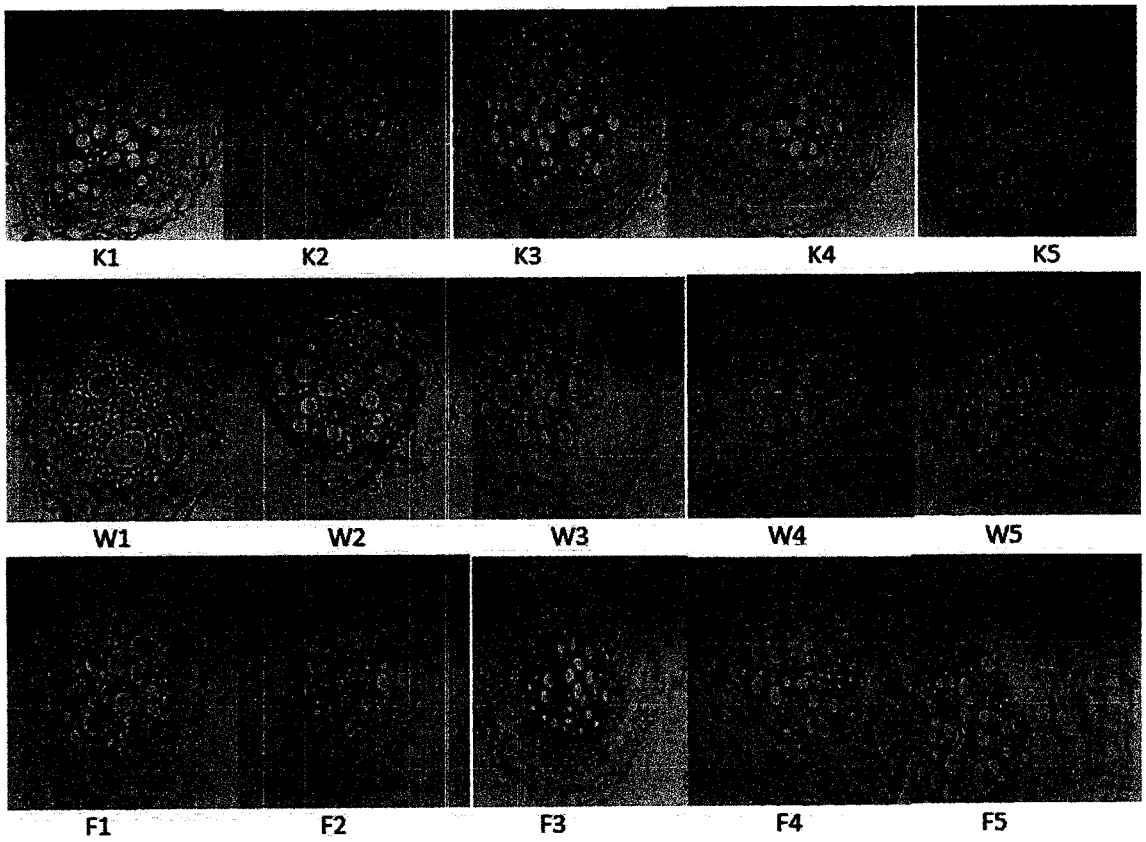


Fig. 1. Treatment of periodic waterloging stress in Tobacco (*Nicotiana tabacum*): A.Waterlogging condition and B. Flooding condition.



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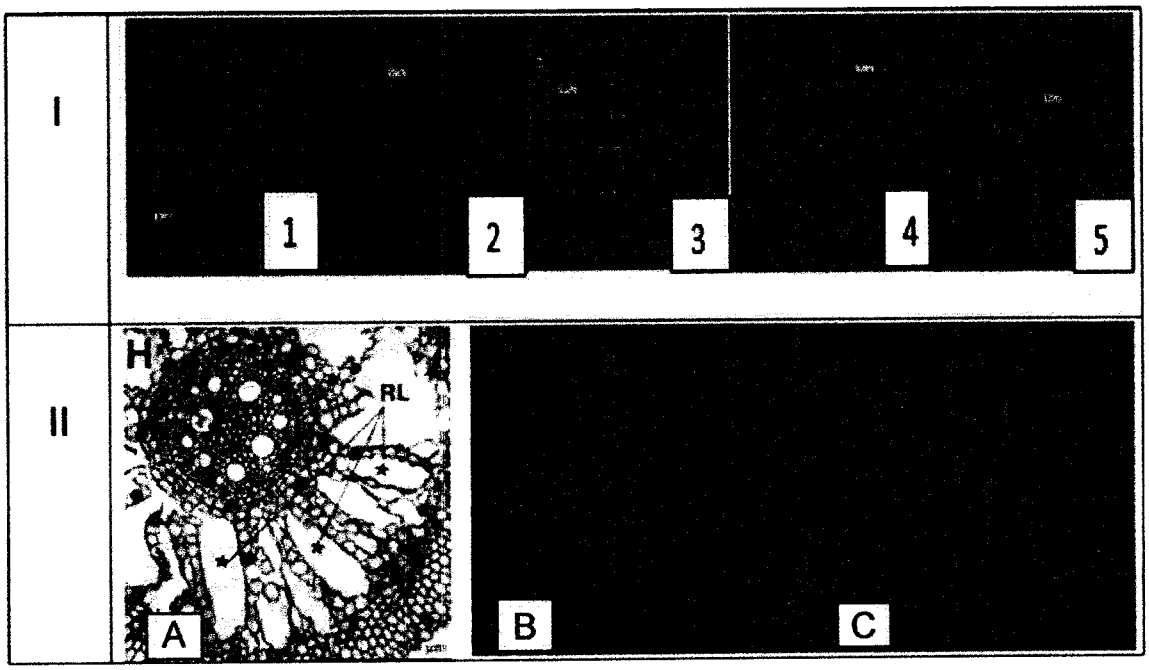

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Table 1
Various root anatomical characters of Tobacco Varieties under periodic waterlogging stress.

Parameter	Treatment	Tobacco Plant Varieties				
		Palakean	Somporis	Marakot	Srumpung	Manilo
Root Diameter (μm)	W0	1356,9 ± 1,48ab	784,47 ± 0,05ab	1618,37 ± 0,36ab	1302,8 ± 0,23ab	1638,77 ± 0,27ab
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	F1	1256,57 ± 0,22ab	846,4 ± 0,42ab	1470,33 ± 0,26ab	1421,4 ± 1,40ab	1670,13 ± 0,07ab
Stele Diameter (μm)	W0	484,13 ± 3,75ad	616,93 ± 0,36ad	981,7 ± 0,14ce	904,57 ± 0,11be	715,57 ± 0,91ce
	W1	833,33 ± 0,17be	562,83 ± 0,12ad	1016,57 ± 0,44ce	658,8 ± 0,21ad	892,67 ± 1,14ad
	F0	348,13 ± 0,19ad	721,67 ± 0,22ad	672,8 ± 0,13ad	842,07 ± 0,46ae	733 ± 0,37ad
	F1	760,07 ± 0,13bd	433,73 ± 0,49ad	751,33 ± 0,68ad	800,2 ± 0,60ad	1332,9 ± 0,52ce
Epidermal thickness(μm)	W0	71,43 ± 0,03bd	107,7 ± 0,05bd	171,9 ± 0,07bd	168,5 ± 0,02bd	177,4 ± 0,04bd
	W1	41,13 ± 0,33ac	28,23 ± 0,09ac	45,43 ± 0,19ac	35,53 ± 0,05ac	66,87 ± 0,23bd
	F0	71,43 ± 0,22bd	107,7 ± 0,10bd	171,9 ± 0,01bd	168,5 ± 0,12bd	177,4 ± 0,12bd
	F1	30,23 ± 0,01ac	23,73 ± 0,05ac	37,93 ± 0,06 ac	32,13 ± 0,09ac	31,23 ± 0,07ac
Cortex thickness (μm)	W0	158,2 ± 0,24ade	65,53 ± 0,17ad	79,13 ± 0,62ad	110,33 ± 0,17ad	155,8 ± 0,16ad
	W1	244,47 ± 0,21bde	42,57 ± 0,12ad	140,83 ± 0,37ad	108,9 ± 0,37ad	224,6 ± 0,04bde
	F0	86,27 ± 0,05ad	157,97 ± 0,22ade	201,7 ± 0,24bde	257,83 ± 0,58bde	225,37 ± 0,45bde
	F1	156,4 ± 0,39ade	155,27 ± 0,22ade	300,8 ± 0,61ce	249,53 ± 0,28bde	514,53 ± 1,14ce
Endodermal thickness (μm)	W0	156,03 ± 0,31bd	51,07 ± 0,20ac	227,03 ± 0,63bd	85,2 ± 0,22acd	120,5 ± 0,13ad
	W1	141,2 ± 0,21ad	50,17 ± 0,02ac	126,87 ± 0,63ad	172,73 ± 0,59bd	111,47 ± 0,15acd
	F0	44,67 ± 0,07ac	122,23 ± 0,10ad	92,27 ± 0,13acd	92,73 ± 0,34acd	207,7 ± 1,03bd
	F1	55,03 ± 0,34ac	45,4 ± 0,11ac	88,33 ± 0,04acd	53,9 ± 0,18ac	168,97 ± 0,44bd
Xylem thickness(μm)	W0	20,3 ± 0,02ab	30,8 ± 0,07ab	28,8 ± 0,03ab	28,73 ± 0,05ab	28,17 ± 0,03ab
	W1	29,7 ± 0,06ab	25,23 ± 0,05ab	36,5 ± 0,08ac	29,87 ± 0,05ab	30,47 ± 0,04abc
	F0	41,9 ± 0,04ac	35,33 ± 0,01ac	31,1 ± 0,03ab	30,37 ± 0,05ab	21 ± 0,06ab
	F1	35,37 ± 0,06ac	29,57 ± 0,01ab	36,9 ± 0,12ac	29,23 ± 0,01ab	34,67 ± 0,02ac
Xylem Diameter (μm)	W0	99,97 ± 0,03ad	116,8 ± 0,01bd	108,87 ± 0,02bc	135,27 ± 0,02bd	87,97 ± 0,18ac
	W1	121,17 ± 0,01bd	97,4 ± 0,02ac	148,1 ± 0,01bd	102,33 ± 0,07ad	116,2 ± 0,17ad
	F0	92,77 ± 0,02ac	102,37 ± 0,01ad	90,87 ± 0,01ac	102,27 ± 0,01ad	102,37 ± 0,01ad
	F1	119,53 ± 0,05bd	76,97 ± 0,03ac	93,6 ± 0,01ac	98,27 ± 0,02ac	87,03 ± 0,02ac
The number of aerenchyma cells	W0	3 ± 0,00ac	1 ± 0,01ab	2 ± 0,02ab	2 ± 0,01ab	3 ± 0,01ac
	W1	8 ± 0,05ac	5 ± 0,03ab	4 ± 0,02ab	5 ± 0,01ab	6 ± 0,02ac
	F0	2 ± 0,01ab	2 ± 0,02ab	1 ± 0,01ab	2 ± 0,01ab	1 ± 0,01ab
	F1	12 ± 0,01ad	10 ± 0,02ad	11 ± 0,02ad	8 ± 0,03ac	7 ± 0,00ac
Aerenchym Cell Length (μm)	W0	34,55 ± 0,06ac	41,4 ± 0,07ad	33,67 ± 0,05ac	45,62 ± 0,06ac	48,97 ± 0,03ac
	W1	115,16 ± 0,04be	97,23 ± 0,09bd	93,37 ± 0,03bd	123,17 ± 0,03be	80,93 ± 0,10ad
	F0	75,2 ± 0,02ad	45,32 ± 0,01ac	42,12 ± 0,03ac	82,45 ± 0,10ad	67,82 ± 0,02ad
	F1	137,76 ± 0,23be	138,63 ± 0,06be	166,1 ± 0,13bf	160,1 ± 0,45bf	168,17 ± 0,34be

Description: 1. numbers followed by the same letters in the same row and column for the measured parameters do not significantly different by Tukey Test at 5%; 2. Treatment Code: W0: Control 1; W1: Waterlogging; F0: Control 2; F1: Flooding

Periodic waterlogging stress treatment with a total 14 days was divided into 7 days in waterlogging conditions and 7 days under flooding conditions.

2.2. Sample preservation tobacco's roots

Root samples were firstly washed before being used for further analysis. Samples were prepared as ± 2 cm size. Subsequently, samples were fixed in FAA solution (formalin: acetic acid: 95% alcohol = 50 ml: 50 ml: 900 ml for every 1 l of solution) in a desiccator tool. Hydration process is then performed in a desiccator for 3 × 30 min. The FAA solution was then removed, and the sample was stored in a 70% alcohol solution [2].

2.3. Observation of root anatomical structure

The cross-sectional root anatomical structure was prepared using modified paraffin method. Procedures of the modified paraffin method used in this study were: (1) gradual dehydration with alcohol; (2) redehydration; (3) immersion through paraffin: dehydrant 1: 1;(4) Embedding;(5) Cutting using microtome;(6) Staining; (8) mounting using entelan. Root anatomical observation was conducted using light microscope with a camera Olympus CX 21 OPTILAB. Quantitative observations of root anatomical roots include total root diameter, stele diameter, epidermal thickness, cortical thickness, endodermic thickness, xylem thickness, xylem diameter, aerenchyme cell count and aerenchyme cell length. The data were analyzed by analysis of variance two way followed by Tukey test.

Acknowledgments

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Transparency document. Supporting information

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The 8th International Conference on Global Resource Conservation (ICGRC)
Biology Department, Faculty of Mathematics and Natural Sciences, University of Brawijaya
Jalan Veteran 169, Malang 65145, Indonesia
Website: http://icgrc.ub.ac.id; Email: icgrc.ub@gmail.com

Our Ref: 154/VI/ICGRC/2017

Malang, 20 June 2017

Subject: **The ICGRC 2017 Invitation and Notification of Abstract Acceptance**

Dear Mrs. Tutik Nurhidayati,

On behalf of the organizing committee, we are glad to inform you that your abstract entitled:

"Response Morphology and Anatomy of Tobacco (Nicotiana tabacum L.) Plant On Waterlogging Stress"

has been accepted for **Oral Presentation** and you are invited as one of the presenters in this coming ICGRC 2017 that will be held on 19-20 July 2017 at UB Guest House, University of Brawijaya, Malang.

We are looking forward to see you in Malang.

Thank you very much.
Yours sincerely,

ICGRC
Asst. Professor Nia Kurniawan, D.Sc
Chairman of the 8th ICGRC 2017

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THE 8TH INTERNATIONAL CONFERENCE ON GREEN TECHNOLOGY

FACULTY OF SCIENCE AND TECHNOLOGY

MAULANA MALIK IBRAHIM STATE ISLAMIC UNIVERSITY OF MALANG

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Acceptance Letter

Dear Tutik Nurhidayati,

We are pleased to inform you that your paper, entitled ***Profile of Protein Levels Some Tobacco Varieties (Nicotiana tabacum L.) on Waterlogging Stress***, meets our presentation qualification (in terms of writing structure and scope of this conference) for **the 8th International Conference of Green Technology** and can be processed to the next steps.

The committee needs to have confirmation from you that you will be able to present your article in our conference at 7th October 2017 and that you will be able to present your paper in the provided 15-minute time slot during the conference. The paper should be 8-10 pages and implementing the template provided by the committee.

Please notify your confirmation to us no more than a week after this letter of acceptance is sent. If you do not send the confirmation within the determined time, your 15-minute time slot will be allocated to another presenter.

We would like you to submit your presentation file (MS PowerPoint or PDF file) to us before 3rd October 2017 so we can give you feedback regarding the time limit of your presentation based on your slide.

We are looking forward to hearing from you.

Best regards,
The Committee of 8th ICGT

Ari Kusumastuti, M.Pd, M.Si

Response Morphology and Anatomy of Tobacco (*Nicotiana tabacum* L.) Plant On *Waterlogging* Stress

Tutik Nurhidayati[1)], Selfrina Puri Wardhani[1)], Hery Purnobasuki[2)], Sucipto Hariyanto[2)], Nurul Jadid[1)]
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Abstrac

Has conducted research morphological and anatomical responses of some varieties of tobacco plants to *waterlogging* stress. Parameters measured were morphology, anatomy and plants sensitivity index. Results were analyzed by Two Way ANOVA followed by Tukey's test. The results show that *waterlogging* stress can reduce of growing tobacco plants that include decrease in plant height with the lowest value of 15.6 cm, root length reduction to the lowest value of 4.6 cm and plant dry weight reduction to the lowest value of 0.26 gr. But the inundation stress can increase the number of adventitious roots with the highest value is 18.33. In addition *waterlogging* stress can lead to the formation of aerenchyma tissue. Based on the sensitivity index showed that plant varieties that are resistant to waterlogging stress are varieties Kemloko 3 (index value of 0.03), varieties of Paiton 2 (index value of 0.18), and the varieties Kemloko 2 (index value of 0.42).

Keywords: *Response, anatomy, morphology, waterlogging stres, sensitivity index, Nicotiana tabacum L.*

Response morphology and anatomy of tobacco (Nicotiana tabacum L.) plant on waterlogging

Tutik Nurhidayati, Selfrina Puri Wardhani, Hery Purnobasuki, Sucipto Hariyanto, Nurul Jadid, and Desy Dwi Nurcahyani

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Response Morphology and Anatomy of Tobacco (Nicotiana tabacum L.) Plant on Waterlogging

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Abstract. This study has conducted research on morphological and anatomical responses of some varieties of tobacco plants to *waterlogging* stress. Parameters measured were morphology, anatomy, and plants sensitivity index. Results were analyzed using two-way ANOVA followed by the Tukey test. The results show that *waterlogging* stress can reduce the growth of tobacco plants, including a decrease in plant height with the lowest value of 15.6 cm, root length reduction to the lowest value of 4.6 cm and plant dry weight reduction to the lowest value of 0.26 gr. But *waterlogging* stress can increase the number of adventitious roots with the highest value of 18.33. In addition, *waterlogging* stress can lead to the formation of aerenchyma tissue. The sensitivity index showed that plant varieties that are resistant to waterlogging stress are the varieties Kemloko 3 (index value of 0.03), varieties of Paiton 2 (index value of 0.18), and the varieties Kemloko 2 (index value of 0.42).

Keywords: Response, anatomy, morphology, waterlogging stress, sensitivity index, Nicotiana tabacum L.

INTRODUCTION

Tobacco is a plantation crop and economically valuable. Tobacco plants live in a dry climate. Tobacco plants require dry land conditions for 2–3 months after planting to harvest leaves and for the ripening process[1].
One risk of tobacco cultivation in Indonesia is the high rainfall can cause *waterlogging*. *Waterlogging* is an abiotic environmental stress that lowers the growth and productivity of plants. *Waterlogging* is a cause of hypoxia or anoxia in stress[2]. *Waterlogging* stress occurs causing the plant roots to become anaerobic conditions.

Plants in a stressed condition will try to survive by way of adaptation. Plants are able to live and grow in waterlogged soil conditions through the adaptation of the anatomy, morphology and metabolic mechanisms[3]. *Waterlogging* stress can cause concentrations of the hormone ethylene and abscisic acid will increase[2]. An increase in the hormone ethylene will induce the IAA and promotes the formation of adventitious roots. In addition, ethylene will induce the death of cells in the root cortex, which in turn form aerenchyma at the root[4].

In this study, five varieties of tobacco plants (Kasturi 1, Kemloko 2, Kemloko 3, Paiton 2 and Prancak N-1) are treated for *waterlogging* stress 50%, 75% and 100% of the field capacity. This study aims to determine the effect of *waterlogging* on morphological and anatomical responses of some varieties of tobacco plants.

EXPERIMENTAL DETAILS

Time and Location of the Study

The study was conducted from March until May 2015 at the Green House Urban Farming ITS and Laboratory of Plant Bioscience and Technology Department of Biology ITS.

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Methodology

The planting medium (1 kg soil: compost 1 kg: NPK 1 gram) used polybags watered until saturated. The media were further allowed to stand for 3 days until there was no dripping water. The planting medium was weighed for wet weight and dry weight. Wet weight was weighed after no water dripped from the polybag. The dry weight was weighed after planting medium oven at 100° C for 24 hours until there was a constant weight[5]. Field capacity is calculated using the formula:

$$W = \frac{Tb - Tk}{Tk} x100\%$$

Information:
W = Capacity Field
Tb = Wet Weight the Planting Medium
Tk = Wet Weight the Planting Medium

Preparation of Seed Tobacco Plant at Treatment *Waterlogging* Stress

Tobacco seed was germinated by way of sowing in small polybags containing growth media for 4 weeks. At the age of 4 weeks after germination, the seedlings were transplanted into polybags containing growing media. Then planting was conducted for 40 days[6]. After 40 DAP (days after planting), we performed the control *waterlogging* test, 50%, 75% and 100% of the field capacity. *Waterlogging* stress was conducted over 10 days.

Morphological observation

Morphological observation of height, root length, plant dry weight, and the number of adventitious roots.

Anatomy Observation (Aerenkim)

Adventitious roots of tobacco plants are cut transversely. Cross sections were placed on a slide and dripped with safranin, which was then covered with a glass lid. The mixture was then observed under a light microscope at a magnification of 100×[7].

The determination of sensitivity index plant

The sensitivity index was measured against the dry weight of plants. The *waterlogging* stress sensitivity index (S) was calculated following the equation[8] namely:

$$S = (1-Yp/Y) / (1-Xp/X)$$

Remarks:

S = Sensitivity Index *Waterlogging* Stress
Yp = The Average Value of Variety With *Waterlogging* Stress
Y = The Average Value of Variety Without *Waterlogging* Stress
Xp = The Average of all varieties with *Waterlogging* Stress
X = The Average of all varieties without *Waterlogging* Stress

The criteria for determining the level of inundation tolerance is:

S < 0.5	= Tolerant (T)
0.5<S<1.0	= Medium Tolerant (MT)
S > 1.0	= Sensitive (S)

Research Design and Data Analysis

This study uses a completely randomized design with factorial a pattern consisting of two factors: the variety and high of *waterlogging*. Analysis of the results of the two-way ANOVA was followed by the Tukey's test to see the real difference in every treatment.

RESULTS AND DISCUSSION

The Influence of *Waterlogging* Stress on the Plant's Height

Based on the results of two-way ANOVA it is known that *waterlogging* stress factors affect high plant with $p = 0.000$ ($p < 0.05$). The results of the ANOVA test were followed by Tukey's test that gives results that the *waterlogging* stress factor has a significant effect on plant height of five varieties of tobacco plants

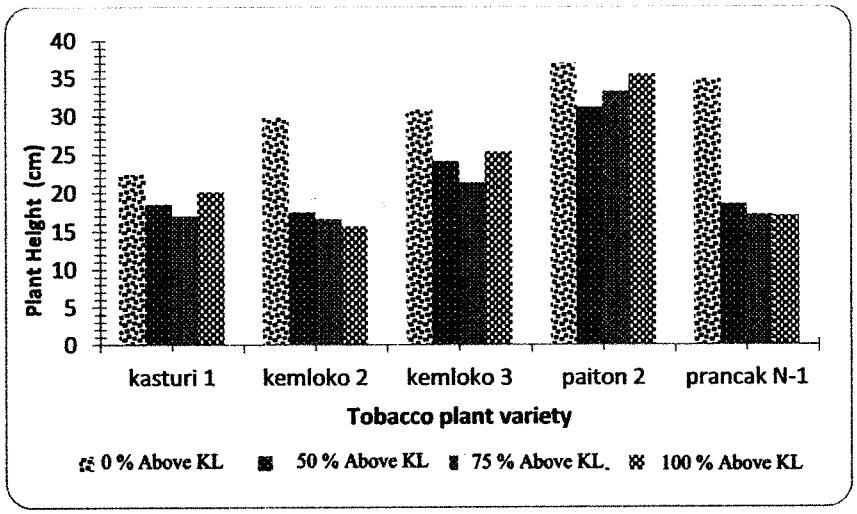

FIGURE 1. High-fifth plant varieties of Tobacco plants after a given *waterlogging stress*

Based on Figure 1, it is known that *waterlogging* stress affects a plant's height in five varieties of tobacco plants. Tall tobacco plants tend to decline when treated with *waterlogging* stress. This is because, with the *waterlogging* stress conditions, the synthesis of the hormone ethylene in plants will increase. Ethylene hormone is a hormone auxin synthesis inhibitor (elongation of the apical meristem) and hormone cytokinin (cell division)[9]. The synthesis of hormones auxin and cytokinin hormone synthesis is inhibited by the presence of ethylene increases, causing the elongation and cell division process to be blocked. For that reason, the presence of the *waterlogging* stress condition can cause growth inhibition of the plant height[10].

The Influence of *Waterlogging* Stress on the length of the Root

Based on the results of two-way ANOVA it is known that stress *waterlogging* factors affect root length with a value of $p = 0.000$ ($p < 0.05$). The results of the ANOVA test followed by Tukey test show that *waterlogging* stress significantly affects root length of the five varieties of tobacco plants.

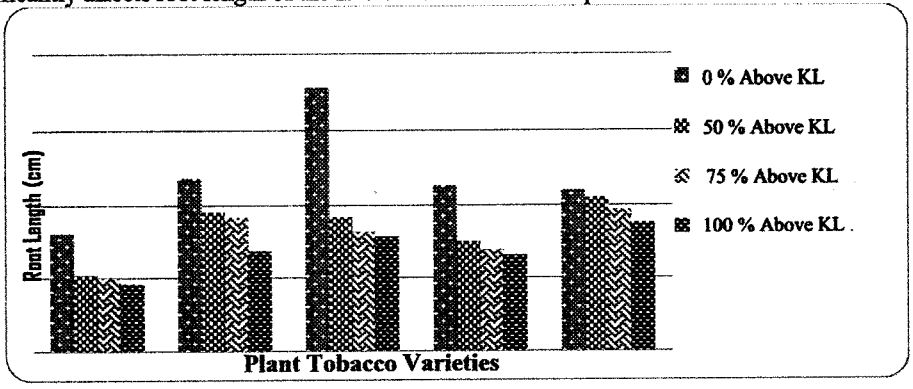

FIGURE 2. The length of the roots of the five varieties of Tobacco plants after a given *waterlogging* stress

As illustrated in Figure 2, it can be seen that the length of the roots of tobacco plants tended to decline when treated with *waterlogging* stress. Long roots decreased in the given conditions of *waterlogging* stress showed that in plants that experienced pool of hypoxia or anoxia, cleavage or extension of the root cells is inhibited resulting in decreased root length[7].

The Influence of *Waterlogging* Stress on a Plant's Dry Weight

Based on the results of two-way ANOVA it is known that stress *waterlogging* factors affect the dry weight with a value of $p = 0.000$ ($p < 0.05$). The results of the ANOVA test followed by the Tukey's test indicate that stress *waterlogging* factors significantly affect the dry weight of the five varieties of tobacco plants.

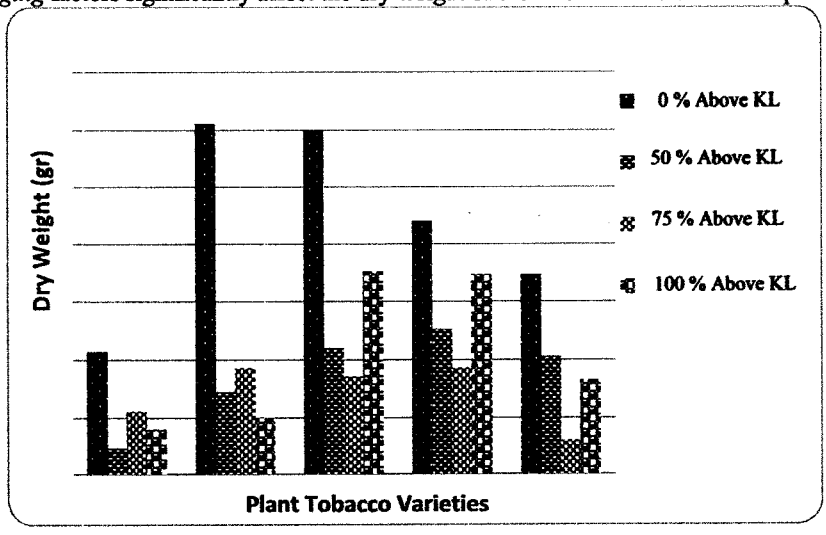

FIGURE 3. The dry weight of the five varieties of Tobacco plants after a given *waterlogging* stress

Figure 3 shows that *waterlogging* stress can decrease the dry weight of plants. This is because the state of hypoxia or anoxia stress occurring in the root zone causes the roots ability to absorb water and nutrients to be inhibited. The inhibition of absorption of water and nutrients causes the supply of water and nutrients to the plants to be low, which makes the dry weight of plants low in *waterlogging* stress[2].

The Influence of *Waterlogging* Stress on the Number of Root Adventitious

Based on the results of two-way ANOVA it is known that *waterlogging* stress factors affect a number of adventitious roots with $p = 0.000$ ($p < 0.05$). The results of the ANOVA test followed by the Tukey's test that gives results that *waterlogging* stress factors significantly affect the number of adventitious roots five varieties of tobacco plants.

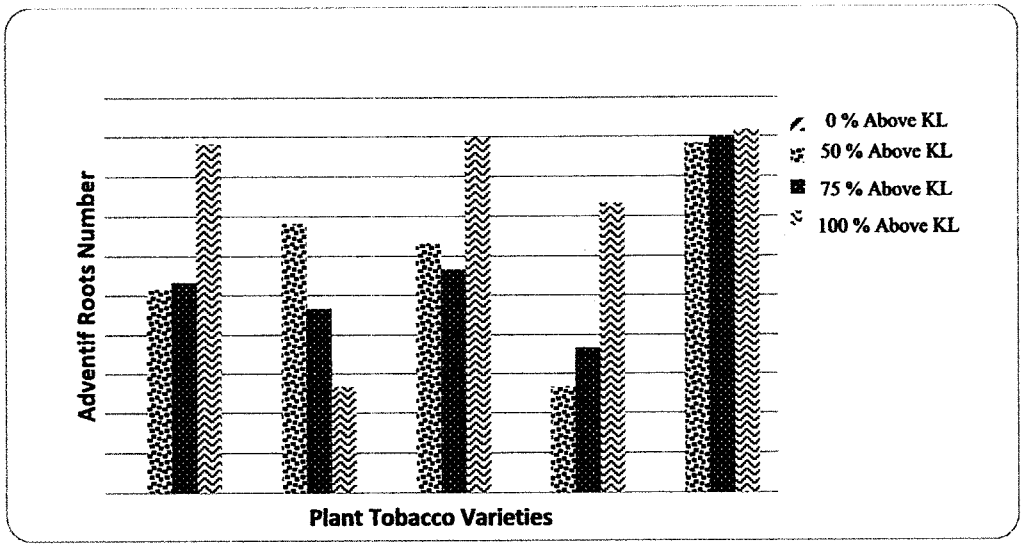

FIGURE 4. Number of adventitious roots five varieties of tobacco plants after being *waterlogging* stress

Figure 4 illustrates that the presence of *waterlogging* stress can trigger the formation of adventitious roots. This suggests that plants in *waterlogging* stress conditions perform morphological a response in the form adventitious root formation in order to survive the environmental conditions of flooding. Adventitious root formation occurs when the original root system is damaged and unable to perform its function as a supplier of water and minerals needed. Furthermore, for energy efficiency then developed root systems customized namely adventitious roots to replace the primary root system damage. The hormone auxin is involved in the formation of adventitious roots. Diffusion of auxin to the root of the lack of oxygen to be slow and auxin accumulates at a meeting with root shoots (shoot-root) in which the adventitious roots are formed[12].

The Influence of *Waterlogging* Stress on The Formation of Aerenchyma

Aerenchyma is a parenchymal tissue of the cavity between the large cells and serves as a store of air. Aerenchyma formation indicates that the plants were seized with a waterlogging response – aerenchyma, the formation to cope with conditions of hypoxia or anoxia. In the *waterlogging* stress condition, ethylene hormone synthesis increased. This is the effect of the hormone ethylene in the process of the formation of aerenchyma. Ethylene causes cortical cells synthesize cellulase enzymes that hydrolyze cellulose and partly causing the cell wall degradability. The lost cortical cell protoplasts and disappeared into aerenchyma tissue. Ethylene triggers cell death and cell destruction at the root cortex. Space previously occupied by these cells would be a vacuum that is used to transfer oxygen[13]. Below is a figure of an aerenchyma network in tobacco plants' adventitious roots.

FIGURE 5. Aerenchyma the adventitious roots of Tobacco plants (eps = epidermis, ko = cortex, en = endodermis, ste = stele, an asterisk (*) = aerenchyma)

Sensitivity Index Tobacco Plants

TABLE 1. The observation of the sensitivity index of the tobacco plant by plant dry weight parameters

Varietas	Plant Sensitivity Index								
	Waterlogging 50%			*Waterlogging* 75%			*Waterlogging* 100%		
	S	MT	T	S	MT	T	S	MT	T
Kasturi 1	1,41			1,52			1,61		
Kemloko 2	1,26				0,97				0,42
Kemloko 3		0,68		1.03					0,03
Paiton 2		0.57			0,83				0,18
Prancak N-1	1,33			1,46			2,04		

Note: S = sensitive, MT = medium tolerant, T = tolerant

Based on Table 1, it can be seen that the varieties that are most tolerant to *waterlogging* stress (100% above field capacity) are the varieties Kemloko 3, Paiton 2 and Kemloko 2. It is influenced by habitat suitability. Kemloko 3 and Kemloko 2 have a native habitat in the rainfed land, while Paiton 2 grows in paddy fields so that all three of these varieties have a high resistance to stress waterlogging compared with Kasturi 1 and Prancak N-1. Kasturi 1 has a native habitat on dry land while Prancak N-1 grows on the ground with a dry climate in Sumenep Madura so that the two varieties were sensitive when stress is placed on the location of waterlogging.

CONCLUSION

The results show that *waterlogging* stress can reduce the growth of tobacco plants that include a decrease in plant height with the lowest value of 15.6 cm, root length reduction to the lowest value of 4.6 cm and plant dry weight reduction to the lowest value of 0.26 gr. But the *waterlogging* stress can increase the number of adventitious roots with the highest value of 18.33. In addition, *waterlogging* stress can lead to the formation of aerenchyma tissue. The sensitivity index indicates that plant varieties that are resistant to waterlogging stress are the varieties Kemloko 3 (index value of 0.03), varieties of Paiton 2 (index value of 0.18), and the varieties Kemloko 2 (index value of 0.42).

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Responses of Periodic Waterlogging Stress In Some Varieties *Nicotiana tabacum*

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Abstrak

Waterlogging Stress can cause a crop production decreased, mainly on terrestrial plants such as tobacco. It is based on the existence of global climate change that shifts the crop and harvest season. Thus, it can increase the losses of farmers. This study aims to investigate the response of Nicotiana tabacum which is gripped by a pool of periodic review of aspects such as the emergence of adventitious roots morphological and physiological aspects such as total leaf chlorophyll. The method in this research is to use four varieties of tobacco plants (Jepon Pelakean, Jinten, Manilo and Morakot) which was given periodic waterlogging stress in the vegetative phase with waterlogging stage for five days, followed by partial submergence during the next five days. Data were analyzed using One-Way Annova with a level of 95% to examine the differences that occur between varieties. In all aspects of morphological parameters (plant height, stem diameter, leaf width and the number of adventitious roots), Pelakean Jepon varieties have better average growth of all three varieties of another test. While the physiological aspects of a decline in chlorophyll content in all varieties of tobacco plants by stress puddle. The decline in chlorophyll content was highest in Manilo varieties.

Key Words: Waterlogging Stress, Nicotiana tabacum, Chlorophyl, Periodic

INTRODUCTION

Tobacco plants is one of the important trading commodity in the world, including Indonesia and a very important role in the national economy [1]. Global climate change, especially on agriculture sector in Indonesia has a significant effect on tobacco plant productivity. Productivity decreases due to damage caused by waterlogging [2]. This is based on the steady and significant increase in flood events over the past six decades [3].

Waterlogging or flooding often used to describe the different situations in which excess water can range from saturated soil water conditions (soil waterlogging) until the formation of the water column causing a thorough immersion in plants (complete submergence) [4]. When flooded, high saturated soil by water and resulted in a drastic reduction in the rate of gas exchange of oxygen into the water 104 lower to air [5]. Oxygen is required to maintain aerobic respiration in submerged tissue [6]. The survival of plants under these conditions depends on physiological, morphological and metabolic adaptations [7].

Morphological responses generally carried out by the plant, including the formation of adventitious roots and an increase in plant height. This resulted in increased biomass aerial organs, namely the trunk organs [8]. The morphological response in plants to facilitate the distribution of oxygen to the tissues were soaked through aerenchyma tissue [9].

Stresses waterlogging soak up part of the shoot, resulting in reduced external carbon dioxide levels were followed by a decline rate of photosynthesis [10]. This condition is exacerbated by the changes in stomatal conductance [4] and a decrease in chlorophyll content [11].

therefore it is necessary to stress puddle on some commercial varieties of tobacco plants are often cultivated, such as varieties of Jepon Pelakean, Jinten, Manilo dan Marakot. It is intended to determine the response, especially from the aspect of morphology and physiology

Metodology

1. Spreading the seed

Seeding is done in mixed media tray with vermicompos and coco peat (1: 1). Furthermore, the growth medium was added NPK (5 g / l medium) and Ridomil solution (0.5 grams / liter of water). All the ingredients are thoroughly mixed growth media. Seeds spread in the media tray evenly. Once the seeds are spread evenly, the tray is closed and stored indoors. Checks carried out on the amount of water on the tray and conditions of seed development to rupture seed (germinated) marked white seed color change on the surface of the media. The seed germination process on the tray is done for 15-20 HSS (Day After Pickling) until it meets the pricking criteria, ie the appearance of 3-4 leaves.

2. Pricking

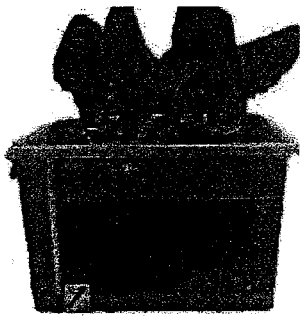
Pricking done on seeds that have appeared 3-4 leaves. Pricking is done in a tray with 72 holes (6x12). The composition of the media tray with the media with the media to spread the seeds on a tray, the media mix vermicompos and coco peat (1: 1) x Growth seedlings in tray HSS conducted during 20-25 days.

3. Displacement in polybag

Preparation of polybag media using a mixture of compost medium and charcoal husk (2: 1). Both were mixed until blended. Transplanting from tray to revoke polybag with relaif uniform seed size manually. Subsequently seedlings were given NPK fertilizer as much as 3 grams / polybag [12]. The growth of seedlings in polybag conducted during 60-70 days.

4. Giving Waterlogging Stress

Seeds that have reached the age of 60-70 HSS with moroflogi 4-5 leaves, then do stress puddle in a plastic container measuring 40 cm x 30 cm x 20 cm. Each container is filled with 3 polybags for the same variety. The treatment of the first stage with a puddle of water to fill the container as high as 13 cm to the achievement of the condition of the roots submerged by water saturated soil (soil waterlogging) for 5 days. The water level should continue to be kept 13 cm.



Stress Waterlogging treatment First Stage

The treatment of the second stage adding a waterlogging of water in the container to soak in part stems and leaves 1-2 first (partial submergence). The second stage is also carried out for 5 days, bringing the total provision waterlogging stress for 10 days.

Stress Waterlogging treatment Second Phase (*Partial submergence*)

The morphological data were collected on the parameters of plant height, stem diameter, leaf width and the number of adventitious roots. Plant height is measured from the soil surface to the tip of the highest growing point using a ruler. The parameters for the diameter of the stem are measured for the diameter of the stem from the base of the stem using a digital caliper. The width of the leaf is measured as the maximum diameter of the imaginary circle which can be placed anywhere in the leaf. Measurement of leaf width using a ruler on a leaf surface is the widest. Adventitious roots appear on both nodes and segments, usually branched and have a bright white color that is different from the fiber roots. The number of adventitious roots is calculated without the inclusion of a branch.

Physiological data collection includes the total measurement of leaf chlorophyll. The method used for total chlorophyll is the extraction of modified acetone. Extraction results were analyzed using a spectrophotometer at wavelengths 663 and 645 nm. All processes are done in dark conditions. Total chlorophyll content is calculated by the formula:

$$\text{Klorofil Total} = 8{,}02\ (A.663) + 20{,}2\ (A.645)\ \text{mg/L}$$

The experimental design was using Completely Randomized Design (RAL) with 4 varieties namely Jepon Pelakean, Jinten, Manilo dan Marakot each having 3 replications. Data were analyzed using ANNOVA One Way (one factor). If there is a real difference, then proceed with Tukey Test 95% level.

Results and Discussion

Morphological responses generally carried out by the plant, including the formation of adventitious roots and an increase in plant height. This resulted in increased biomass aerial organs, namely the trunk organs (Grimoldi et al., 1999 in SC et al., 2015). The morphological response in plants to facilitate

the distribution of oxygen to the tissues were soaked through aerenchyma network (Laan et al., 1990; Colmer, 2003a). Some other morphological response observed is the withered leaves (wilting), premature aging of the leaves, and the increase in diameter of the rod (Dennis et al., 2000 in SC et al., 2015). The observed morphological parameters plant height, stem diameter, leaf width and the formation of adventitious roots

1. The Impact of Periodic Waterlogging Stress At Plant Height

Plant height is a common response that occurs in plants that waterlogging stress. This can occur as a result of the interaction of plant hormones, such as abscisic acid (ABA), Gibberellins (GA) and Ethylene (Jackson, 2008)

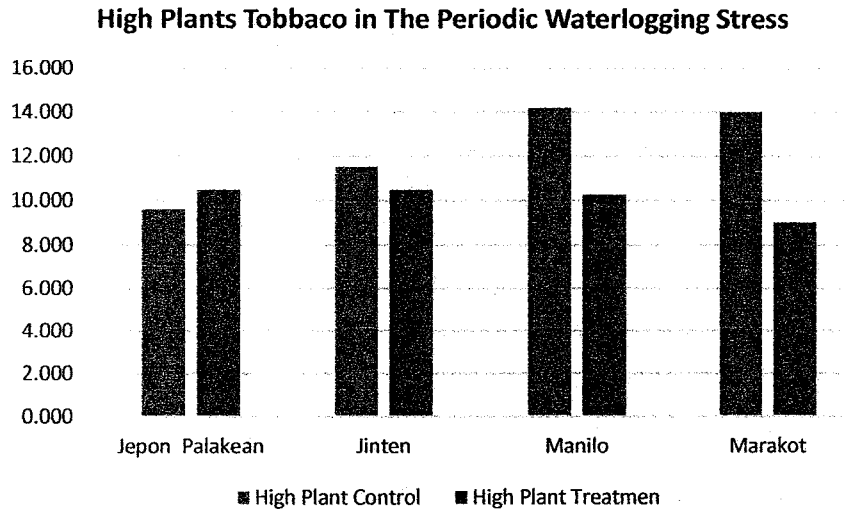


Figure 4.1 Average Plant Several Varieties of *N. tabacum* After Giving Periodic Waterlogging Stress

Based on the figure 1 is known that among the four varieties of the test being treated, only varieties Jepon Pelakean showing increased plant height. Meanwhile, three other test varieties showed the opposite response, namely the decrease in plant height than the control. Basically, each variety has a different response based on internal interactions within the plant hormone. This also happened in previous research by Dubois et al. (2011), which showed that between rice varieties with one another showed different responses in higher plants. The difference in response was also due to factors hormones involved in drought stress, such as ethylene, ABA and GA.

Jepon Pelakean varieties show a general response crops that occurs in the waterlogging stress, ie stem hypertrophy than control. The adaptation response mechanism of the stem hypertrophy requires the biosynthesis of gibberellin (GA) hormones that depend on the availability of absorbent acid hormone (ABA). Basically, the accumulation of ethylene increased under hypoxic conditions lead to the regulation of ABA levels decrease through the inhibition of the expression of 9-cis-epoxycarotenoid dioxygenase and via activation of ABA into phaseic acid solution. This certainly causes a decrease in endogenous ABA content required in the stimulation of the expression of GA 3oxidase (an enzyme which catalyze changes into a bioactive gibberellins / GA1). In addition, an increase in the endogenous hormone ethylene will result in a lower pH in the apoplast which helps stimulate the cell wall loosening as the steps needed to initiate cell elongation (Jackson, 2008). Hypertrophy rod in conditions of hypoxia / anoxia, with regard to the composition of a white spongy tissue that has a volume between large gas space (Armstrong et al., 1994). The network is a secondary aerenchyma formed externally

from felogen and is a homologue of cork tissue (Shimamura et al, 2010; Teakle et al., 2011). Its role is to increase the gas space allowing for an increase in gas movement between submerged and non-submerged tissues (Teakle et al., 2011). As for some of the species that have been studied previously and may develop on the stem hypertrophy is *Lythrum salicaria* (Stevens et al., 1997), *Lotus uliginosus* (James & Sprent, 1999), *L. tenuis* (Striker et al., 2005), *Glycine max* (Shimamura et al., 2010; Melilotus Siculus, 2010 and Teakle et al., 2011).

On the contrary, the varieties Morakot, Manilo and jinten showed the opposite response Jepon Pelakean varieties. Three varieties are showing plant height less than the control. In the Dubois et al. (2011) which provides waterlogging stress between two rice varieties shows different responses. Lowland-rice varieties show different responses from deepwater-rice varieties that undergo stem lengthening in general. Lowland-rice varieties have increased the expression of genes that suppress the formation of GA SUB1A through increased repressor SLR1 and SLRL1. Thus, it can be assumed that varieties of Marakot, Manilo, and Jinten also increase the expression of the SUB 1A-like gene that plays a role in GA biosynthesis. Then SUB 1A-like gene expression repressor increase SLR1 and SLRL1 which suppresses the synthesis of GA. This then raises the morphology of the three varieties trunk is smaller than the control.

2. Impact of Waterlogging Stress on Stem diameter

The increased stem diameter response cropped to waerlogging stress is a strategy of adaptation through the formation of aerenchyma, thereby increasing the diameter of the stem. The formation is controlled by elevated endogenous ethylene hormones in submerged tissue and triggers the expression of Xyloglucan Endotransglycosylase (XET) (Drew et al., 2000).

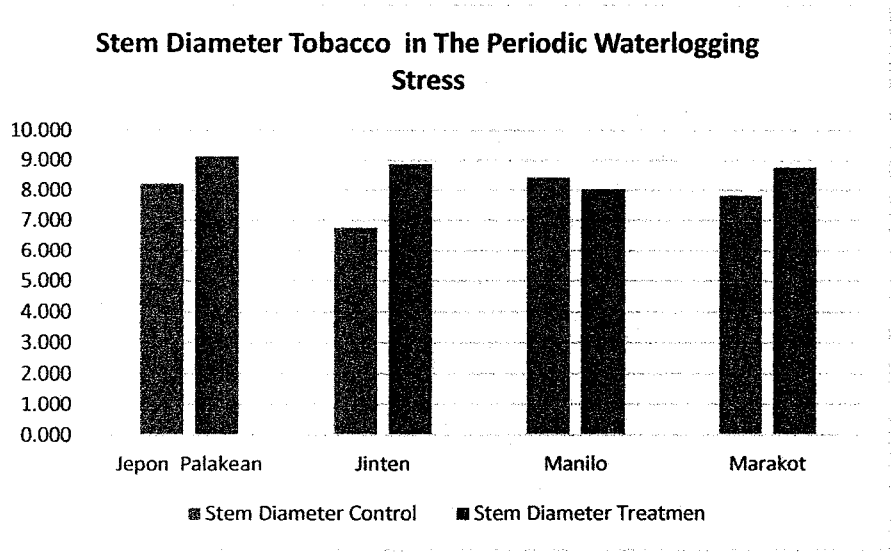


Figure 5 Changes in Stem Diameter Several varieties of *N. tabacum* Having given Periodic waterlogging Stress

Based on a variety Jepon Image Pelakean, jinten , and Morakot has a trunk diameter greater than the control plants. While on Manilo varieties showed a decrease in stem diameter than the control plants.

Aerenchyma formation shown by the plant in response to the current low oxygen availability, which helps facilitate the diffusion of gases (such as O2) between the root hypoxia / anoxia with aerialnya environment (Garthwaite et al., 2008; Pistelli et al., 2012). Under hypoxic conditions, the establishment of aerenchyma involve cell death caused by an increase in the rate of ethylene biosynthesis (Jackson, 1985; He et al., 1994; Gunawardena et al., 2001). Aerenchyma tissue is also

formed on the organ in which the stem form an interconnected system from the leaf to the root tip (Mommer et al., 2007; 2006).

Aerenchyma formation is triggered by an increase in sensitivity of the endogenous hormone ethylene (He et al., 1992). In waterlogged conditions, the concentration of O2 subambien stimulate the production of ethylene in the roots, which then accumulates in them and induces Program Cell Death (PCD) or death of cells in the cortex (He et al., 1996). In addition, the increased ethylene also resulted in the increase of cellulase enzymes in roots play a role in cell lysis (Drew, 1992; Grineva and Bragina, 1993; He et al., 1994). Then there is also an increase in the activity of pectinase and xylanase whose role is to degrade the cell wall polysaccharides (Lasanthi et al., 2007; Liu et al., 2005; Sat and Sachs., 1996).

Previous research on the species *Zea mays*, waterlogging stress induces the expression of genes that encode XET1 XYLOGLUCAN formation ENDOTRANSGLYCOSYLASE (XET1), which is an enzyme that plays a role in cell wall loosening (Peschke and Sachs, 1994; Saab and Sachs, 1995, 1996). Lack of oxygen triggers the formation of XET1 on the primary roots, mesokotil and coleoptile. XET1 induced by hypoxic conditions associated with the formation of aerenchyma (Saab and Sachs, 1996).

3. Impact of Periodic Inundation Puddle Against Leaf Width

Plants respond to waterlogging stress conditions by altering hormonal balance and abnormal growth that may occur due to over-production of ethylene (Grichko and Glick, 2001; Saleem et al., 2007). Abnormal growths occurring during hypoxia / anoxia, especially in terms of leaf width is the decrease in leaf width. This condition is also exacerbated by nutrient deficiencies that primarily affect plant growth under conditions of waterlogging stress (Steffens et al., 2005).

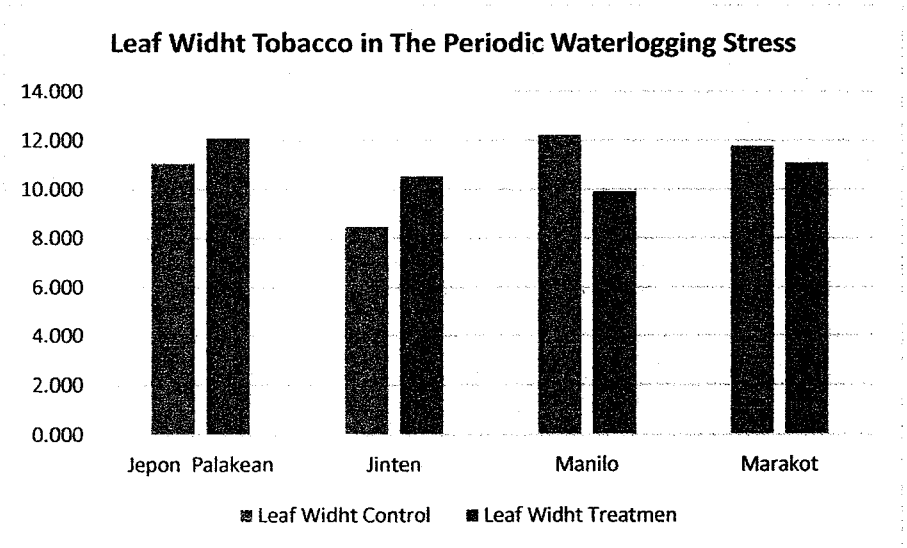


Figure 6. Changes in Leaf Width of Varieties *N. tabacum* After Giving Periodic waterlogging Stress

Based on Figure 6 it can be seen that Jepon Pelakean and Jinten varieties, showed a larger leaf width than control plants. Unlike the case with varieties of manilo and marakot that have a smaller leaf width than control plants. Differences in leaf width of four varieties of the test, also caused by the leaf morphological character of each variety. Of the four varieties of test used, jinten varieties that have leaves more oval than the other three varieties. So that in Figure 6 looks average the lowest compared

to the third control other varieties. However, when compared to controls, decreased varieties Manilo highest average leaf width, of 2.3. This can be attributed to the different hormonal interaction in every variety, especially hormone cytokinin decreased in conditions of hypoxia / anoxia (Burrows and Carr, 1969).

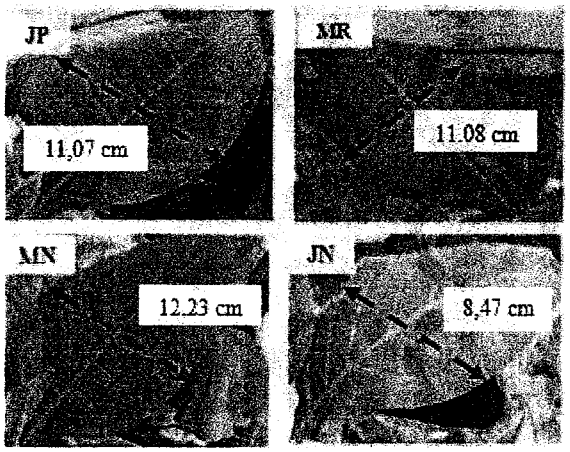


Figure 7. Morphology of the Fourth Leaf Test Variety on Control (Personal Documentation, 2015): Description (JP = Jepon Pelakean varieties MR = Marakot Varieties MN = Manilo Varieties JN = Varieties of Jinten)

Within one day of waterlogging stress , cytokinin hormone concentrations reported to have very low concentrations in the xylem vessels. This is as a result of reduced synthesis of cytokines at the root, which is caused by the condition of hypoxia / anoxia. Thus, it causes the inability roots in cytokinin transported to the aerial organs of the plant, the leaves and stems (Burrows and Carr, 1969).

As a result, a low cytokinin in aerial organs, particularly the leaf organs can block cell division and affect the average width of the leaf treatment that is narrower than the controls. Cytokinins are synthesized in the root tip meristem (Short and Torrey, 1972), which became the earliest decline in metabolic activity and cell death compared to other networks (VanToai et al., 1995). Related to this, it is thought that there is a decrease in expression of the IPT gene encoding isopentenyl transferase in initiation and limiting the pathway stage of cytokinin biosynthesis. When compared with control, it is suspected that IPT gene expression occurs normally, and results in normal growth of leaf organ.

4. Impact of Waterlogging Stress Cause Against Adventitious Rooting

The emergence of adventitious roots is one common response of plants that were flooded to get oxygen supply is declining, especially in the root. Ethylene and Auxin interact to control the formation of adventitious roots. Effect of Ethylene on each different species, most of which have a positive impact form adventitious roots (Roy et al., 1972; review in De-Klerk et al., 1999; Clark et al., 1999; Negi et al., 2010) , but a small number of species exhibit inhibitory responses (Coleman et al., 1980; Nordstrom and Eliasson, 1984), or have no significant impact (Batten and Mullins, 1978).

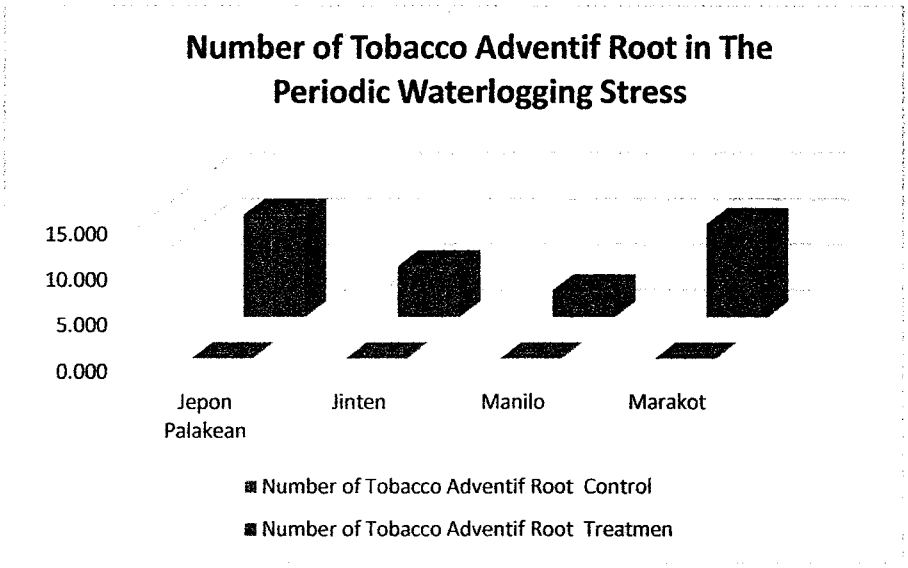


Figure 8. Average Number of Adventitious Root Occurrences Some Varieties *N. tabacum* After Given Periodic Waterlogging Stress

Interaction of these hormones are also thought to be different in the four varieties tested in the formation of adventitious roots, both in terms of time and number of the establishment. On the fourth day of treatment, several varieties have raised their adventif roots to the soil surface, namely Jepon Pelakean and Marakot varieties. While on the varieties of Jinten and Manilo appear on days 5-6 treatments. In terms of number, the highest incidence of adventif root occurs in Jepon Pelakean varieties are 11 pieces each individual, then Marakot Varieties which have an average of 10 root of each individual adventif. While in the control plants, did not bring the root of adventif, this is because *N. tabacum* plants grown normally belonging to terrestrial plants (unsaturated soil saturated by water).

Currently hypoxic conditions, an increase in activity of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, which is a major enzyme in the biosynthesis of ethylene (He et al., 1994; Sairam et al., 2008). In a previous study by Visser et al. (1996), the formation of adventitious roots Rumex palustris occur through the role of ethylene which can increase sensitivity to auxin transport and auxin mediates in basipetal from shoots to roots. So that the accumulation of auxin proficiency level, then bring adventitious roots around the base of the stem. (Riov and Yang, 1989; Visser et al., 1996).

Adventitious roots needed to sustain plant growth for a plant submerged (waterlogged) maintaining the continuity of taking nutrients and water (Sairam et al., 2008). Thus, in this study, the emergence of adventitious roots on the fourth test varieties to support growth during the treatment. This is evident in the number of populations that remain until the end of treatment.

5. Impact of Periodic Inundation Cutting on Total Chlorophyll Plant

Plant physiological response to stress inundation is very dependent on the tolerance level of a species. The physiological responses can be stomatal closure, reduction in transpiration and photosynthesis inhibition (Mollard et al., 2008; 2010 in Calorina, 2015). Plants also respond to anoxic conditions by regulating protein synthesis specifically for anaerobic conditions (anaerobic polypeptide / ANPS) (Sachs et al., 1980). Parameteryang observed is the total chlorophyll of leaves

The negative impact of the waterlogging stress is inhibition of leaf growth, biomass reduction, a decrease in stomatal conductance and decrease the amount of chlorophyll a and b in the leaves [4].

The decline has been significant chlorophyll in the older leaves, indicating their rapid degradation of chlorophyll in the leaves.

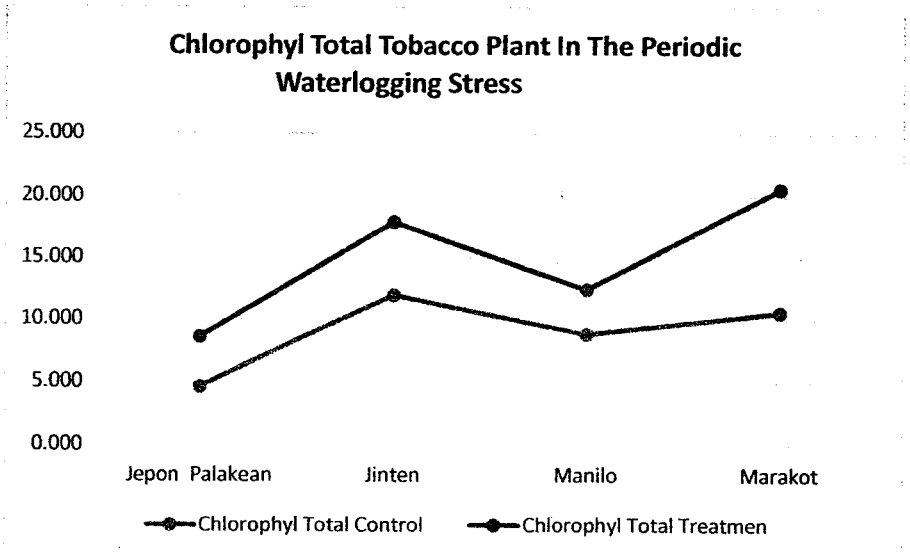


Figure 9. Mean Total Chlorophyll Some varieties of *N. tabacum* After Periodic waterlogging Stress Given Compared to Control Plants

Based on Figure 9, a decline in average total chlorophyll simultaneously on all four varieties of the test due to the provision of treatment. The highest chlorophyll content owned by Morakot varieties under stress conditions inundation, which only decreased by 2.8%, then Jepon Pelakean varieties decreased by 7.6% compared to controls. Meanwhile, the biggest decline in chlorophyll content occurred in Manilo Varieties that is equal to 41.5%, followed by cumin varieties of 33.8% when compared with controls. Range reduction in total chlorophyll in four varieties quite far away, where it can be said that the varieties Morakot and Jepon Pelakean have total chlorophyll better quality than the other two test varieties on this parameter.

Results of the study include decrease in chlorophyll in all varieties tested, showed that a suspected periodic inundation stress treatment induces an increase in the hormone ethylene plant. At the root hypoxia / anoxia, increased ACC least 4 hours soaking conditions (submergence). Conversion of ACC into ethylene oxygen. Then the ACC is transported to the stem section and also the leaves are not stagnant and the ACC is oxidized to ethylene (Bradford and Yang, 1980). The result is an increase in the hormone ethylene content results in the leaves and cause chlorosis in some leaves, which are characterized by a drastic decrease in chlorophyll content (Bradford and Dilley, 1978; Morgan and Drew, 1997). One transcription factor in the biosynthesis of ethylene signaling that EIN3 control gene that positively regulates AtNAP leaf senescence and activate the ABA signaling pathway components. ABA also contribute to the aging of leaves and respond to abiotic stresses which occur (Guo and Gun, 2006; Zhang and Gan, 2012).

CONCLUSION

In all aspects of morphological parameters (plant height, stem diameter, leaf width and the number of adventitious roots), Pelakean Jepon varieties have better average growth of all three varieties of another test. While the physiological aspects of a decline in chlorophyll content in all varieties of tobacco plants by stress puddle. The decline in chlorophyll content was highest in Manilo varieties.

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Profile of Protein Levels And Cholorophyl Content Some Tobacco Varieties(*Nicotiana tabacum* L.) On Waterlogging Stress

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Abstract

Tobacco is a high-value crops that are sensitive to waterlogging stress. Some tobacco varieties have been widely cultivated in Indonesia, including Jepon Mawar, Jepon Banyak and Rejeb. Some species have different abilities to withstand the conditions of waterlogging stress by morphological adaptation, anatomy, physiology, and metabolic pathway changes. Changes in protein profiles is one form of plant defense response to waterlogging stress. Profile proteins experienced upregulation in hypoxia and anoxia conditions when gripped waterlogging stress known as anaerobic polypeptides. In addition to the waterlogging stress will cause the decrease in chlorophyll levels as a result of chlorosis during the stress. The purpose of this research is to know protein profile and chlorophyll content of several varieties of tobacco (Nicotiana tabacum L.): Rejeb, Jepon Mawar and Jepon banyak against stagnant waterlogging stress. The protein profile was analyzed by SDS-PAGE method. While the chlorophyll content was analyzed by spectrophotometric method. The protein profiles expressed from the three test varieties were present in the molecular weight range 85.38-153.33 kDa. Proteins with a molecular weight of 85.38 kDa are thought to have similarities to the Peroxsidase group (BM = 85 kDa) that play a role in the ROS detoxification process. While the chlorophyll content in the three varieties decreased with the increasing of waterlogging stress except on Rejeb varieties treated by 175% and Jepon Mawar varieties on 200% waterlogging stress treatment.

Keywords: Waterlogging stress , *Nicotiana tabacum* L., protein profile and chlorophill

1. INTRODUCTION

Waterlogging of an abiotic stresses which may affect growth and yields. One of the causes of waterlogging is the high rain insensity. The main cause of damage to the plant during the waterlogging is the low oxygen, which indicated the existence of wilting symptoms due to the absorption of nutrients and water that hampered [1]. Waterlogging can also cause the condition of hypoxia and anoxia in the soil. Stress reduces the pool of gas exchange between the plant tissue and air as gas diffusion layer in water 10,000 times slower than in air [4]. Despite the low oxygen availability is an important factor affecting plant growth, soil chemical element content, such as pH and redox potential, is also changing under waterlogging stress conditions and can also affect the survival and growth of plants [5,6

Lack of oxygen stimulates anaerobic fermentation that affect less well on some morphological and physiological processes, such as photosynthesis, energy metabolism, redox potential, gene expression, as well as degradation and protein synthesis [7]. One form of plant responses to abiotic stress is the change in protein expression and post-translational modification of proteins to activate the defense system in the face of stress [8]. The protein profile changes in hypoxia. In addition, also expressed specific proteins that have upregulation in this case is anaerobic polypeptides. The enzyme plays a role in the metabolism of glucose, glycolysis, fermentation [9], hormone synthesis, programmed cell death [10].

Lack of oxygen stimulates anaerobic fermentation that affect less well on some morphological and physiological processes, such as photosynthesis, energy metabolism, redox potential, gene expression, as well as degradation and protein synthesis [7]. One form of plant responses to abiotic stress is the change in protein expression and post-translational modification of proteins to activate the defense system in the face of stress [8]. The protein profile changes in hypoxia. In addition, also expressed specific proteins that have upregulation in this case is anaerobic polypeptides. The enzyme plays a role in the metabolism of glucose, glycolysis, fermentation [9], hormone synthesis, programmed cell death [10]. Reported by Ahzan (2007), found a total of 35 proteins in the roots of tomato plants (*Lycopersicum esculentum*) in response to the waterlogging stress 16 proteins had increased expression and 13 proteins had decreased expression. In maize (*Zea mays*), the treatment of waterlogging stress changes the pattern of protein synthesis in the primary root [12].

Response of the other plant in response to the waterlogging stress is a decrease in the conductance of stomata (Folzer et al., 2006), changes in hormone balance (Else et al., 2001), decreased transpiration and inhibition of photosynthesis rate due to a decrease in leaf chlorophyll content (Cao and Conner, 1999). These responses occur within hours or days, depending on the level of tolerance of plant species (Striker et al., 2005). The decline of chlorophyll and chlorophyll degradation massively occur during leaf senescence, fruit ripening, and also in response to environmental stress (Hortensteiner and Bernhard, 2010). The decline in the chlorophyll content due to intake of nutrients, especially of N low due to damage to the root system due to flooding.

Determination of protein profiles and chlorophyll content of some varieties of tobacco in response to waterlogging stress provide a better understanding of its function in adaptation to stress.

2. RESEARCH METHOD

2.1 Preparation of tobacco seeds

Tobacco varieties seed in this study include var. Rejeb, Jepon Mawar, and Jepon Banyak. The seeds were germinated in germination media containing compost and chaff (2:1). Germination was carried out for 15 days after seeding (15 das). Germinated seeds were then pricked (until seedlings aged 54 das) and transferred *pottray*.

2.2. Planting tobacco seeds in polybag

Tobacco seedlings 54 das were transferred to non-perforated polybags. Planting medium used in this procedure was compost and chaff with a ratio of 4:1. Each polybag contains 1 kg of planting medium. 5 gr NPK fertilizer was applied for each plant. Pest and disease control was done using insecticides organtrin 1.5 ml / L. The control carried out at the time of old tobacco plants (15 dap). Tobacco plants were grown for 3 weeks (21 days after planting (dap).

2.3. Field capacity measurement

Measurements of field capacity were conducted to determine the volume of the water in the treatment of *waterlogging stress*. Planting media that were placed in polybag were watered until the water passed through the media. The media was subsequently left to stand for 3 days until there were no the dripping water occurred. Furthermore, the media were directly weighted. Meanwhile, dry weight media were measured by placing the planting medium in the oven at 100° C for 24 hours until reaching a constant weight. Field capacity is calculated using the formula:

$$W = \frac{Tb - Tk}{Tk} \text{ X } 100\ \%$$

Information:

W : Field Capacity
Tb : Wed Weight (Gram)
Tk : Dry Weight (Gram)

2.4. Stress Waterlogging Treatment

Waterlogging stress treatments were carried out using 21 dap tobacco plants. *Waterlogging stress* treatment used in this study were 100%, 150%, 175% and 200%. These treatments were applied during 10 days. The volume of water was maintained for 10 days of treatment.

2.5 Sample Preparation (Protein Extraction)

Protein profile analysis using protein electrophoresis method. Plants that have been taken are washed with distilled water. A total of 0.250 grams of leaf organs were washed with phosphate buffered saline (PBS) pH 7.4. The organs were homogenized with cold mortar and added 500 µl of protein extract buffer. Homogenate is inserted in a 1.5 ml tube and centrifuged at 10000 rpm at 4 ° C for 10 minutes. The pellet is removed and the supernatant is inserted a new 1.5 ml tube then stored at -20 °C. For electrophoresis used 12.5% separating gel and 5% stacking gel.

2.6 Sample Preparation (Chloropyl Content)

Leaf chlorophyll was measured by spectrophotometric method using the modified Hall and Rao method in Priadi (2014). Leaves weighing 0.1 grams were crushed in porcelain porcelain and dissolved in 10 mL of 95%

methanol for 24 hours in the dark. Furthermore, the extract was filtered with Whatman no. 1 and The solution is introduced into the test tube and covered with aluminum foil. Then the 3 ml solution was introduced into the cuvette and measured its absorbance by spectrophotometer at 645 and 663 nm wavelengths. Chlorophyll levels can be expressed in mg of chlorophyll per gram of material (Yoshida et al., 1976 in Rachmawati and Retnaningrum, 2013)). Total chlorophyll content is calculated by the formula (Harborne, 1987):

2.7 Parameters of observation

Parameters include the observation of profile protein and content chlorophyll.

2.8 Data Analysis

The data chlorophyll content obtained were analyzed using ANOVA Two Way followed by *Tukey* test and descriptive to analysis provile protein.

3. RESULTS AND ANALYSIS

3.1 Protein Profile Jepon banyak Variety

Based on the results of SDS-PAGE analysis Jepon banyak varieties are shown in Figure 1 shows that a protein with a molecular weight of 153.33 kDa decreased expression represented by the thickness of the protein band with increasing level of flooding. Protein band with a molecular weight of 119.51 and 103.98 kDa expressed only in the treatment of 100% waterlogging stress and degradation as indicated by the loss of protein bands on the treatment of 150%, 175% and 200%. The protein band with a molecular weight of 90.48 kDa is expressed only in the concentration of 175% and 200% waterlogging. While the protein with a molecular weight of 85.38 kDa was uniformly expressed on all the treatment of waterlogging stress. The results show that there are some proteins that degrade or decrease expression when the waterlogging stress. In addition there are also proteins that are only expressed at high waterlogging rates such as proteins with molecular weight of 90.48 kDa. The weight of expressed protein molecules differs across all treatments ranging from 85.38-153.33 kDa.

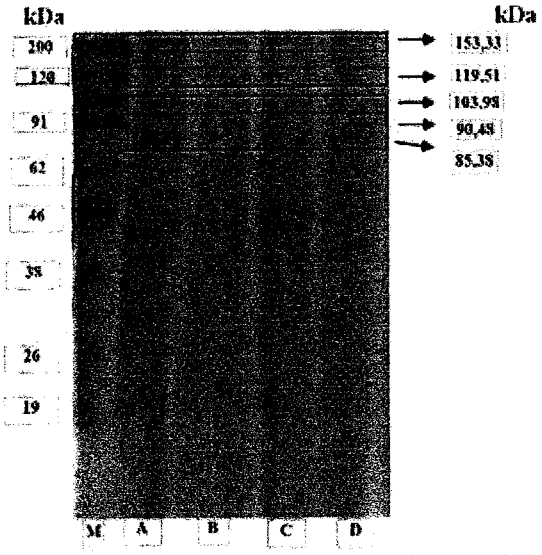

Figure 1. Results of Analysis of SDS-PAGE Profiles of Jepon Banyak Variety Proteins. : (M) Marker; (B) 100% Waterlogging stress above the field capacity; (C) 150% Waterlogging stress above the field capacity (D) 175% Waterlogging stress above the field capacity (E) 200% Waterlogging stress above the field capacity.

Research on tomato plants (*Solanum lycopersicum*) treated with waterlogging for 14 days showed that expressed proteins ranged in molecular weight 50-110 kDa [21]. The 50-55 kDa molecular weight protein is a member of the Rubisco Large-Subunit (RLS). The 60-61 kDa molecular weight protein is Rubisco Binding Protein (RBP), a molecular weight protein of 93-95 kDa which is an ATP-dependent protease belonging to serine protease (Clp-P), and a protein with a molecular weight of 110 kDa is a protein Rubisco Activase (RA). Based on the results of identification, these proteins play a role in the process of photosynthesis. Rubisco's expression decreased in total dissolved protein during response to the waterlogging stress. Rubisco has two functions that act as carboxylases that mediate CO2 assimilation and as oxygenase in catalyzing the early photorespiration stage [22]. In addition, the presence of waterlogging stress can also induce the formation of ROS (Reactive Oxygen Species) which may lead to the degradation of Rubisco subunit and Rubisco Activase.

3.2 Protein Profile Rejeb Variety

In Rejeb varieties, occurring protein with molecular weight of 153.33 kDa increased expression in the treatment of waterlogging stress 175% as indicated by the band thicker than other treatments. Protein with a molecular weight of 119.21 and 103.98 kDa was also expressed on the previous varieties. These proteins are expressed in the treatment of 100% and have been degraded in the treatment of 150%, 175%, 200%. In addition, there is an increased expression of the protein bands with molecular weight of 85.38 kDa when the waterlogging stress treatment 175%, it is seen from the thickness of the protein bands. Band thickness decreases as 200% waterlogging stress concentration as shown in Figure 2.

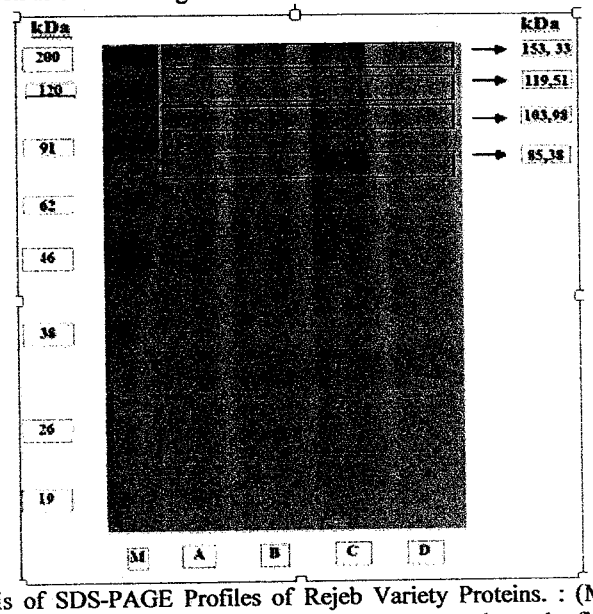

Figure 2. Results of Analysis of SDS-PAGE Profiles of Rejeb Variety Proteins. : (M) Marker; (B) 100% Waterlogging stress above the field capacity; (C) 150% Waterlogging stress above the field capacity (D) 175% Waterlogging stress above the field capacity (E) 200% Waterlogging stress above the field capacity.

According to [23], maize (*Zea mays*) were flooded for 52 hours showed increased expression of a protein with a molecular weight of 85 kDa and 133 kDa. Protein with a molecular weight of 133 kDa initially decreased expression during the initial 4 hours of waterlogging stress, and increased after 28 to 52 hours of waterlogging stress. While the protein with a molecular weight of 85 kDa significantly increased its expression during 52 hours of waterlogging stress. Based on the results of identification, both proteins are included in the peroxidase group that play a role in the physiological functions of plants including plant development, oxidative stress and processes associated with plant cell walls. Peroxsidase also plays a role in the production and detoxification of ROS (Reactive Oxygen species) caused by stress.

Oxidative stress occurs when unbalanced production and neutralization of ROS within cells. High light, heat, pathogen attack, low oxygen levels, and re-aeration after hypoxia phase can increase ROS production [25]. ROS is produced from molecular oxygen through several reduction steps. Anion superoxide (O_2-), hydroxyl radical (OH), singlet oxygen ($^1O_2$) results from the reduction of one or three electrons from oxygen by the reduction energy provided by the electron carriers in mitochondria and chloroplasts [26]. ROS is highly reactive and can cause damage to lipid membranes and proteins [23]. Plants have a protective system to protect mitochondria from overproduction of ROS such as antioxidants (glutathione, ascorbic acid, tocopherol, tannins, ubiquinol, and phenolic acid), ROS scavenging enzymes such as superoxide dismustase (SOD), catalase (CAT), ascorbate peroxidase (APX), and gluthatione peroxidase (GPX). So it can be predicted that an increase in protein expression with a molecular weight of 85.38 kDa in the Rejeb variety (175% stroke) is one form of protection mechanism of oxidative stress that can damage the lipid membrane and other proteins. It is also supported with Rejeb reef morphology data of 175% treatment which showed better growth compared to 100% treatment.

3.3 Protein Profile Jepon Mawar Variety

In Jepon Mawar varieties, also express proteins that appeared in previous varieties as shown in Figure 3, which is a protein with a molecular weight of 119.51 and 103.98 kDa that appears only in the treatment of

waterlogging stress 100%. Protein with a molecular weight of 153.33 kDa stress treatment increased expression as a pool of 200%.

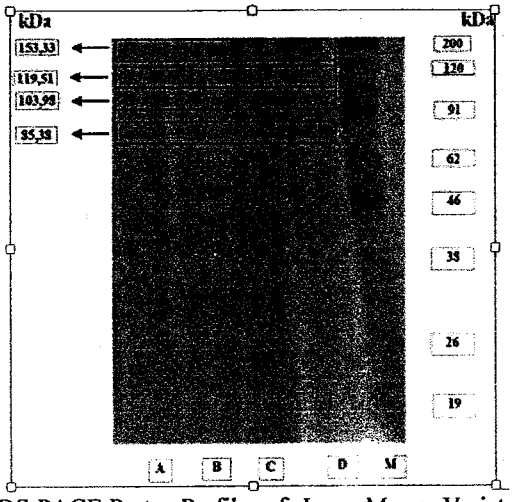

Figure 3. Results of Analysis of SDS-PAGE Proten Profiles of Jepon Mawar Variety : (M) Marker; (B) 100% Waterlogging stress above the field capacity; (C) 150% Waterlogging stress above the field capacity (D) 175% Waterlogging stress above the field capacity (E) 200% Waterlogging stress above the field capacity.

Increased expression also occurs in Jepon Mawar varieties with 175% stress treatment. While the protein with a molecular weight of 85.38 kDa expressed only in the treatment of a pool of 100% stress and degradation or decreased expression with increasing level of flooding. So it can be said that the majority of proteins that appear on the treatment of 100% have been degraded in the other treatment.

4. Chlorophyll Content

The negative impact of the waterlogging stress is inhibition of leaf growth, biomass reduction, a decrease in stomatal conductance and decrease the amount of chlorophyll a and b in the leaves [4]. The decrease in chlorophyll occurs significantly in older leaves, indicating rapid degradation of chlorophyll in leaves near the stagnant roots. Their stress puddle on 3 different varieties show varying levels of chlorophyll content. This is shown in Figure 4.

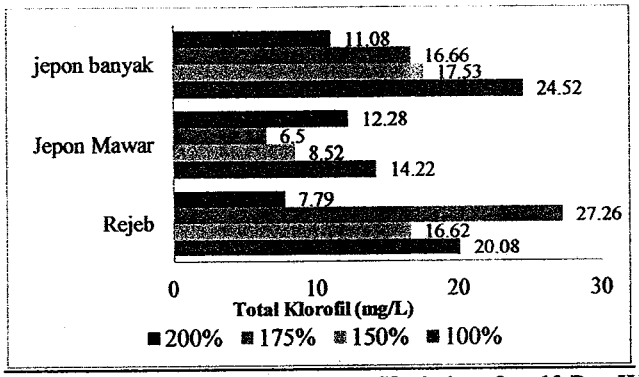

Figure 4. Average Total Chlorophyll Several Tobacco Varieties after 10 Day Waterlogging Stress

According to Fig. 4, it can be seen that the total chlorophyll in the three varieties decreased except in Rejeb varieties treated by waterlogging stress 175% and Jepon varieties on 200% waterlogging stress treatment. The decrease in chlorophyll levels is thought to be caused by the destruction of stagnant roots. Waterlogging stress can cause the death of cells that can inhibit root development and function of the root system such as fetching water and nutrients, especially nitrogen, so that the need for water and N of the canopy is not met. As a result leaves wither and yellow. One of the benefits of N to the plants is for the formation of chlorophyll in the leaves. Chlorophyll degradation can be demonstrated through chlorosis on the leaves that gripped puddle (Pociecha et al., 2008) as shown in Figure 4.

4. CONCLUSION

Stress can alter protein expression inundation of some varieties of tobacco. SDS-PAGE analysis of tobacco leaf protein indicates that there are 5 different proteins expressed in each variety and treatment of waterlogging stress. proteins are expressed differently in all three test varieties contained in the molecular weight range from 85.38 to 153.33 kDa. Varieties Rejeb increase protein expression at a molecular weight of 85.38 kDa in the treatment of 175%, as indicated by the thickness of the protein bands. The protein is degraded in the varieties of roses Jepon treatment 150%, 175% and 200% and expressed the same in all treatment Jepon Many varieties. Protein with a molecular weight of 85.38 kDa allegedly has in common with Peroxsidase group (molecular weight 85 kDa), which plays a role in detoxification of ROS.The chlorophyll content in the three varieties decreased with the increasing of waterlogging stress except on Rejeb varieties treated by 175% and Jepon Mawar varieties on 200% waterlogging stress treatment.

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ABSTRACT

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THEME:

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entomopathogenic fungi associated with scale insect in the field. A collection of fungi isolated from single conidia and its ability to infect selected scale insect. Entomopathogenic fungi pathogenic next on viability and pathogenicity test against scale insect. The results showed that the scale insect attack citrus is kind L beckii and A. aurantii. The highest attack occurred in lowland agro-climate during the dry season by L. beckii with tails and the rising population of 4.2 to 5.5 individuals per 10 cm in the rainy season. The result of selection of entomopathogenic fungi with density 107 conidia/ml with LC 50 within 14 days produced 12 isolates obtained during the dry season and 9 isolates during the rainy season. Viability test results showed that the isolates had viability above 50%, namely SK B4 K, SK D1 K and SB B3 K are respectively 73.6%, 61.6% and 53%, which were collected during the dry season and out of season isolates obtained rain SBW D2 H and SBW D3 BH each with a viability of 77.3% and 78.3%. Pathogenicity test results showed that there were six isolates are known to have a potential for controlling scale insect entomopathogenic fungi namely SBW B2 H, SBW D2 H, SBW D3 BH, SK D1 K, SBW D1 K and SB B3 K pathogenicity which has over 50 % up to 14 days.

Citrus, entomopathogenic fungi, pathogenicity, scale insect, viability

BP-02

Abundance, size distribution, and sex ratio of freshwater crabs *Parathelphusa convexa* in Mengaji River, Central Java, Indonesia

Diana Retna Utarini Suci Rahayu[♥], Agatha Sih Piranti, Anastasia Endang Pulungsari

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The river is one of the freshwater crab habitats, the land use changes will affect the abundance and distribution of biota. The study on the abundance, distribution and sex ratio freshwater crab *Parathelphusa convexa* (De Man, 1879) on the Mengaji river, Banyumas, Central Java, Indonesia was conducted in April to June 2015. The method used in the form of surveys using traps, that trap installed in each station observation of as many as 15 pieces of each station. Total crabs caught as many as 117 individual with a sex ratio of male: female = 1: 0.89. Crab carapace length ranging from 13.75 to 41.42 mm for males and 13.48 to 38.78 mm for females. Carapace width ranged from 13.60 to 48.42 for males and 15.65 mm up to 45.50 mm for females. The total weight range of between 1 to 48 grams for males and 0.8 to 31gram for females. The highest abundance was obtained in the downstream areas as many as 62 individuals. The results showed that at the same size, weight, length and width of carapace *P. convexa* caught in the River Mengaji, Banyumas larger than females

Abundance, distribution, *Parathelphusa convexa*

BP-03

Respon of *Nicotiana tabacum* plant to waterlogging stress during vegetative stage

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Tobacco is one of the important commodity in Indonesia. Some varieties of tobacco cultivated in Indonesia, among others varieties Srumpung, Dixie Bright and Somporis. However, some constraints were found in the cultivation of tobacco is increasing the frequency of rain that caused waterlogging. Waterlogging stress can reduce the growth and productivity of plants. Waterlogging stress condition, the plant will try to survive by adapting morphology, anatomy, physiology, and biochemistry. One tobacco plant responses to waterlogging stress are adventitious root formation and closing of stomata. The purpose of this study was to evaluate the response of some varieties of tobacco plants (varieties Srumpung, Dixie Bright and Somporis) to waterlogging stress. The method used is the provision of treatment of stress puddle with a percentage of 100%, 150%, 175%, and 200% in the third test varieties. The observation form adventitious roots number and the number of stomata open and close.

Adventitious roots, *Nicotiana tabacum*, stomata, waterlogging stress

Diversity of Ecosystem

CO-01

Refining the suitability modeling of sea cucumber (*Holothuria scabra*) by using a fully raster-based data

Bambang Sulistyo[1,♥], Mukti Dono Wilopo[1], Dede Hartono[1], Ully Wulandari[2], Noviyanti Listyaningrum[3]

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[3]Faculty of Geography, Universitas Gadjah Mada. Bulaksumur, Sleman 55281, Yogyakarta

The research was aimed at refining the suitability modeling of sea cucumber (*Holothuria scabra*) by using a fully

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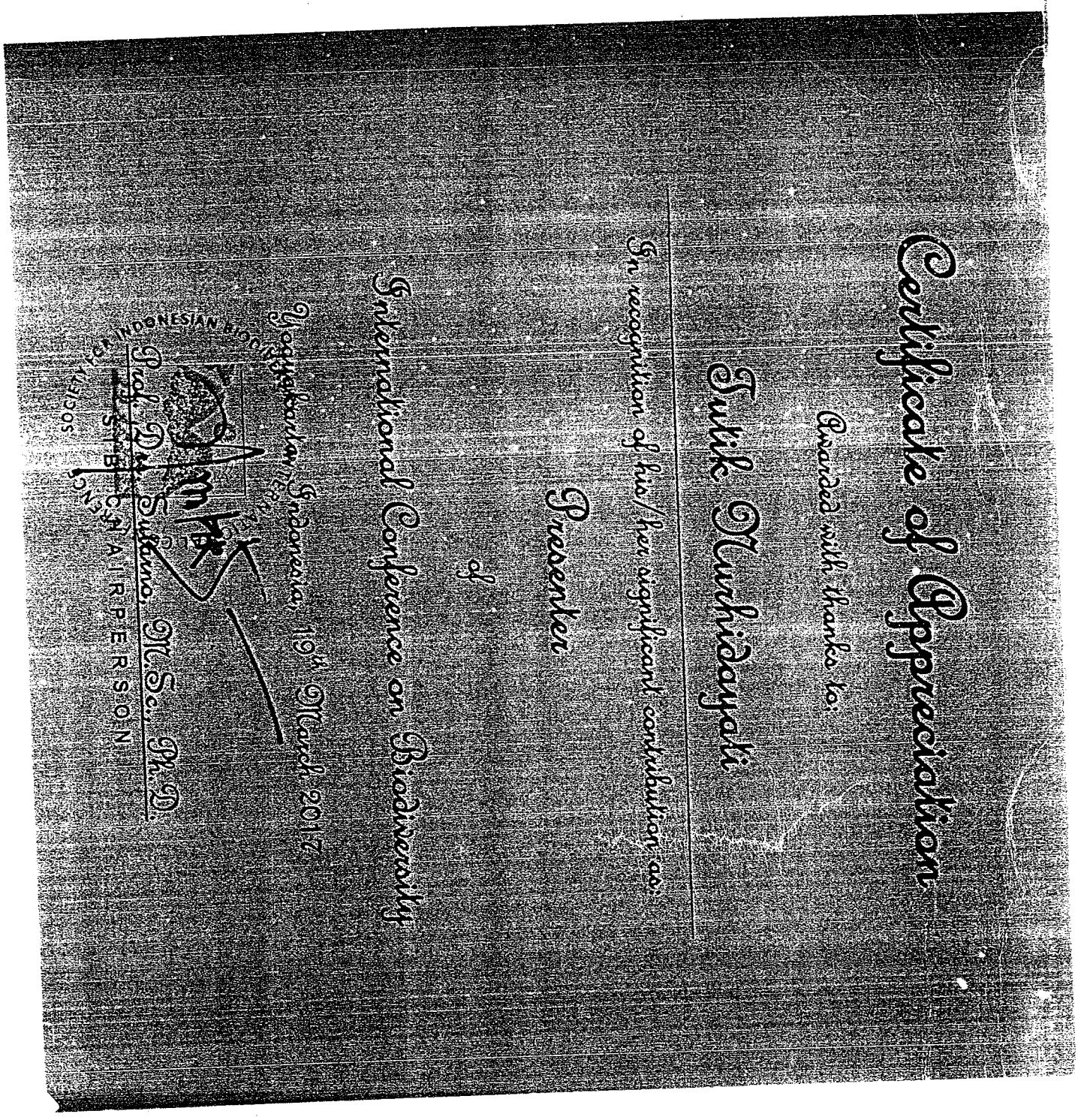

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Respon of *Nicotiana tabacum* plant to *Waterlogging* Stress During Vegetatif Stage

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ABSTRACT

Tobacco is one of the important commodity in Indonesia. Some varieties of tobacco cultivated in Indonesia, among others varieties Srumpung, Dixie Bright and Somporis. However, there are constraints that were found in the cultivation of tobacco is increasing the frequency of rain that caused *waterlogging*. *Waterlogging* stress can reduce the growth and productivity of plants. *Waterlogging* stress condition, the plant will try to survive by adapting morphology, anatomy, physiology, and biochemistry. One tobacco plant responses to stress *waterlogging* are adventitious root formation and closing of stomata. The purpose of this study was to evaluate the response of some varieties of tobacco plants (Srumpung, Dixie Bright and Somporis varieties) to *waterlogging* stress. The method used is the provision of treatment of *waterlogging* stress with a percentage of 100%, 150%, 175%, and 200% in the third test varieties. The observation form adventitious roots number and the number of stomata open and close.

Key words: *Nicotiana tabacum*, *Waterlogging* Stress, Vegetatif Stage, Adventitious, and Stomata

INTRODUCTION

Waterlogging an abiotic environmental stress which can reduce the growth and productivity of plants. *Waterlogging* can be divided into two kinds, the first only plant roots waterlogged (waterlogging), the second is the flooding which is divided into two, namely the entire root to most shoots submerged (partial submergence), and all the plants submerged in water (complete submergence) (Bacanamwo, and Purcell, 1999; Suwarti *et al.*, 2013).

Waterlogging is a cause of hypoxic or anoxic stress in plants. In addition *Waterlogging* result in anaerobic conditions at the plant roots, thus resulting in decreased gas exchange between the soil and air (Hodson and Bryant, 2012). Of the conditions affects the O_2 availability to plant roots and soil microorganisms to be very limited. Oxygen availability is limited to the plant roots led to unstable transport of nutrients and water to the leaf tissue.

Besides *Waterlogging* stress can reduce leaf water potential resulting stomata close. The closure of the stomata cause wilting in plants (Bradford and Yang, 1981) and ultimately reduce the productivity of the plant. According to (Sairam and Ezhilmathi. 2009), the lack of oxygen due to *waterlogging* stress is the limiting factor of growth and productivity of plants.

Plants are exposed to stress such as *waterlogging* of adaptation. Adaptation plant in waterlogged soil conditions through the adaptation of anatomical, morphological and metabolic mechanisms (Pourabdal *et al.*, 2008]. According to (Amico *et al.*, 2001), the initial response of affected plants is *waterlogging* stress stomatal closure quickly. The closing of stomata which cause the plant withers away. Moreover, it also happens reduction of carbon fixation during photosynthesis (Jackson and Drew, 1984], which is caused by stomatal closure (Striker, *et al.*, 2005).

In addition to the *waterlogging* stress, the plants forming adventitious roots that absorbs oxygen (Suwarti, *et al.*, 2013). Some plants are capable of forming adventitious roots as a response to the loss of oxygen. The adventitious roots root replace damaged due to oxygen deprivation stress during *waterlogging* stress. Adventitious roots were formed more effectively in the transfer of oxygen for the roots also form aerenchyma. Adventitious roots can reduce the adverse effects by expanding the *waterlogging* stress rooting area to the air, increasing the aerobic respiration, and oxidize the rhizosphere (Bacanamwo and Purcell, 1999).

The study aims to determine the growth response (the number of adventitious roots, the number of stomata opening and closing) of several varieties of the tobacco plant to *waterlogging* stress.

MATERIALS AND METHODS

Study area

The research was conducted at PT. Sadhana Purwosari Pasuruan East Java and Laboratory of Plant Ecophysiology and Technology Department of Biology, Institut Teknologi Sepuluh Nopember Surabaya East Java, in February to June 2016.

Procedures

1. Preparation of tobacco seeds

Tobacco seed used Srumpung, Dixie Bright and Somporis. Seeds germinate in germination media containing compost and chaff (2:1). Germination carried out for 15 days (15 HSS). Once the seeds germinate carried *pricking* that is transplanting to *pottray*. Planting medium used in the same *pricking* at the time of germination. Pricking done until seedlings 54 HSS.

2. Planting tobacco seeds in polybag

Seedling tobacco plants that have been outstanding 54 HSS transferred to *polybags* not perforated. Planting medium used is compost and chaff with a ratio of 4: 1. Each *polybag* containing 1 kg of planting medium. Tobacco seeds in the media pottray (age: 54 HSS) then planted in polybag media. Fertilization was done at the beginning of planting. NPK fertilizer used is 5 grams per plant. Pest and disease control is done using insecticides organtrin 1.5 ml / L. The control carried out at the time of old tobacco plants (15 HST). Tobacco seeds in polybag media were grown for 3 weeks (21 HST).

3. Measurement Capacity of Field

Measurements of field capacity is made to determine the volume of water giving in the treatment of waterlogging *stress*. Media planted in polybags are watered with water to a trickle. Media subsequently left to stand for 3 days until no the dripping water. Furthermore, the wet weight measured media. To measure dry weight, the planting medium oven at 100 ° C for 24 hours until reaching a constant weight. Field capacity is calculated using the formula:

$$W = \frac{Tb - Tk}{Tk} \times 100\ \%$$

Information:

W = Field capacity
Tb = Wet Weight
Tk = dry Weight

4. Stress Waterlogging Treatment

Stress *waterlogging* treatment carried out on tobacco plants aged 21 HST. Stress *waterlogging* treatment used was 100%, 150%, 175% and 200%. Stress *waterlogging* conducted over 10 days. The volume of water in each treatment is maintained for 10 days of treatment.

5. The observations Roots Adventitious

The observations adventitious roots done by counting the number of adventitious roots that form on each plant (Chen and Miller, 2002).

6. The observations Stomata

Stomata anatomical observations performed by making an incision paradermal using the whole (whole mount) were stained with safranin 1% (Sass, 1951; Damayanti, 2007). A preparation observed under a microscope with a magnification of 400x. the observation preparations done by counting the number of stomata that opens and closes.

Data analysis

The data obtained were analyzed using ANOVA Two Way. A further test using the Tukey test.

RESULTS AND DISCUSSION

Results and Discussion should be written as a series of connecting sentences, however, for manuscript with long discussion should be divided into subtitles. Results should be clear and concise.

A. The number of Adventitious Roots

Adventitious roots emerge from the stem of the plant is submerged and grow horizontally. This is possible as an adaptation mechanism by which new roots formed to replace the original root system has been damaged (Sairam *et al.*, 2008). Treatment stress waterlogging in tobacco plants can promotes the formation of adventitious roots. The number of adventitious roots increased with the percentage of stress waterlogging. The condition is shown in Table 1 and Figure 1.

Table 1 Average The number of Adventitious Roots in the of Stress *Waterlogging* Treatment

Varieties	Stress *Waterlogging* Treatment			
	G1 (100%)	G2 (150%)	G3 (175%)	G4 (200%)
Srumpung	0.00	3.67	6.00	7.67
Dixie Bright	0.00	3.33	6.67	8.67
Somporis	0.00	3.00	5.33	9.33

Note: G1 = Stress *Waterlogging* With Field Capacity 100%; G2 = Stress *Waterlogging* With Field Capacity 150%; G3 = Stress *Waterlogging* With Field Capacity 175%; G4 = Stress *Waterlogging* With Field Capacity 200%.

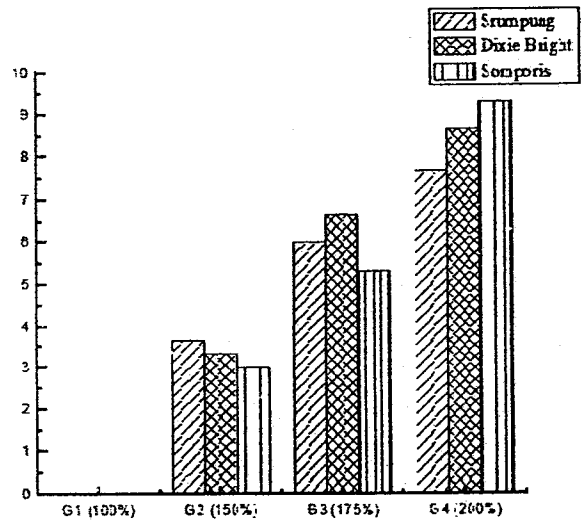

Figure 1. An average Number of Adventitious Roots On All Tobacco Plant Variety at Each treatment Stress *Waterlogging*

B. Number of Stomata

Stomata are the small pores located on abaxial (top) and adaxial (lower side) of plant leaves. Consists of a pair of stomatal guard cells and a few extra cells. Stomata function to regulate carbon dioxide assimilation and transpiration process through turgor changes in guard cells and stomata open and close the hole. It is very important to the global carbon cycle and the water plant's ability to respond to environmental changes (Gan *et al.*, 2010). In this study, the observed number of stomata are open and the number of stomata are closing

B.1 Number of Stomata Opening

The number of stomata open in tobacco plants being treated inundation stress decreased with the increasing percentage of stress *waterlogging*. A decrease in the number of open stomata in plants Srumpung tobacco varieties, Dixie Bright and Somporis. At treatment stress *waterlogging* of 200%, a decrease in the number of open stomata highest in Dixie Bright tobacco plant varieties by 85.14%. Whereas in Somporis varieties decreased the number of stomata open at 74.06% and the Srumpung varieties. The condition is shown in Table 2 and Figure 2.

Table 2. Average Number of Stomata Open in Each Treatment of Stress *Waterlogging*

Varieties	Stress *Waterlogging* Treatment			
	G1 (100%)	G2 (150%)	G3 (175%)	G4 (200%)
Srumpung	6.53	7.67	3.00	2.73
Dixie Bright	6.73	7.00	1.40	1.00
Somporis	6.67	5.47	2.00	1.73

Note: G1 = Stress *Waterlogging* With Field Capacity 100%; G2 = Stress *Waterlogging* With Field Capacity 150%; G3 = Stress *Waterlogging* With Field Capacity 175%; G4 = Stress *Waterlogging* With Field Capacity 200%.

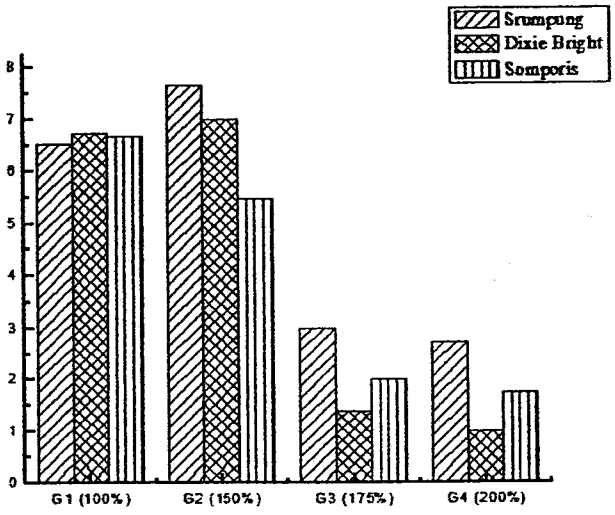

Figure 2. An average Number of Stomata Open at all Tobacco Plant Variety at Each treatment Stress *Waterlogging*

B.2 Number of stomatal closes

The number of stomata are closed in tobacco plants being treated Stress *Waterlogging* increases with increasing percentage of Stress *Waterlogging*. Increasing the number of closed stomata in plants Srumpung tobacco varieties, Dixie Bright and Somporis. At treatment Stress *Waterlogging* 200%, increase in the number of stomata closed-highest in tobacco plants amounted to 85.78% Somporis varieties, Dixie Bright varieties to 81.13% and amounted to 64.30% Srumpung varieties. Such conditions can be shown in Table 3 and Figure 3.

Table 3 Average Number of Stomata Closed on Stress *Waterlogging* Treatment

Varietas	Stress *Waterlogging* Treatment			
	G1 (100%)	G2 (150%)	G3 (175%)	G4 (200%)
Srumpung	3.07 cd	3.00 cd	8.87 a	8.60 a
Dixie Bright	1.27 d	1.87 d	5.07 bc	6.73 ab
Somporis	1.27 d	3.40 cd	5.07 bc	8.93 a

Note: G1 = Stress *Waterlogging* With Field Capacity 100%; G2 = Stress *Waterlogging* With Field Capacity 150%; G3 = Stress *Waterlogging* With Field Capacity 175%; G4 = Stress *Waterlogging* With Field Capacity 200%.

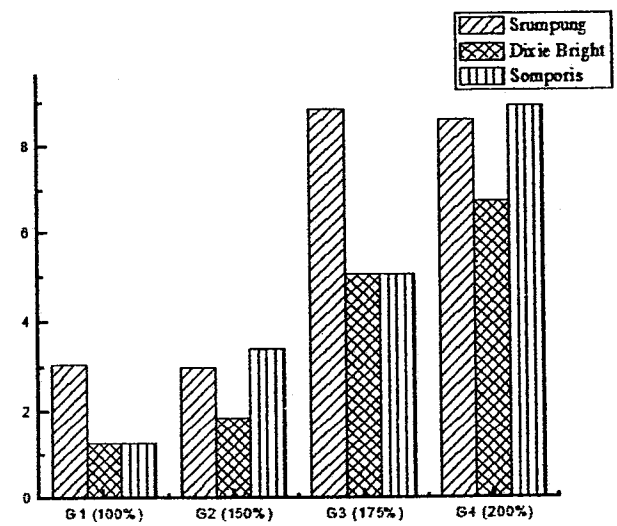

Figure 3. An average Number of Stomata Closes at all Tobacco Plant Variety at Each treatment Stress *Waterlogging*

Discussion

In the treatment of stress waterlogging 200%, Somporis varieties have an average number of adventitious roots largest and followed by Dixie Bright and Srumpung. While in control of all varieties are not formed adventitious roots. The condition shows that stress puddle in tobacco plants can spur the growth of adventitious roots [15]. Adventitious root formation is an adaptation of plants to environmental roots are of oxygen deficiency. At the time the plant in a state of hypoxia (lack of O_2), adventitious roots will form on the upper the root where high oxygen tension. Adventitious roots grow laterally from the base of the main stem. Adventitious roots can reduce the adverse effects by expanding the pool of rooting area to the air, increasing the aerobic respiration, and oxidize the rhizosphere (Bacanamwo and Purcell. 1999). Adventitious roots replacing the damaged main root and more effective in the transfer of oxygen. Adventitious roots needed to sustain plant growth during stagnant and maintaining sustainability taking nutrients and water (Sairam *et al.*, 2008).

Adventitious root formation due to the interaction between the hormones auxin and ethylene (Akhtar and Nazir, 2013). Hormone auxin is involved in the formation of adventitious roots. The role of adventitious roots helps the absorption of water and nutrients to plants, facilitate the diffusion of the final product of the alcoholic fermentation of plants that do not accumulate (Cronk and Fennessy, 2001).

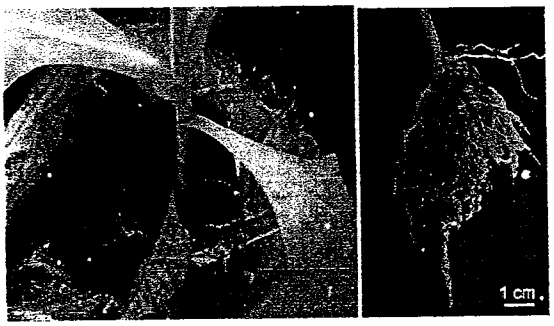

Figure 4. Adventitious roots were formed in the Tobacco Plant Stress Treatment Given Stress Waterlogging of 200%

Early response of plants that were flooded were closing stomata. Plant responses to frequently increase the pool of abscisic acid on the leaf that play a role in stomatal closure. So the presence of abscisic acid inhibits the growth of leaves. Selan their ethylene can also cause stomata closes. The condition is caused by ethylene and abscisic acid. Both of which can cause changes in the cell that disrupts the protective membrane get out-the entry of water and ions. The incident will increase the concentration of CO2 and cause stomata closes (Riche, 2004).

Stomatal guard cells open for be filled with water and push the inner wall of the stomata to move closer. Guard cells can grow long, especially the outer wall, to expand outwards. Then, the inner wall will be attracted by mikrofibil resulting stomata open (Salisbury & Ross, 1995). Besides decreasing the number of stomata are open is also due to the response of plants to stress *waterlogging*. In the inundation stress occurs the excess water in the rooting environment. The presence of excess water in the rooting environment cause physiological dryness in the plant body. To maintain the water supply plant transpiration rate decreased by reducing the number of stomata open.

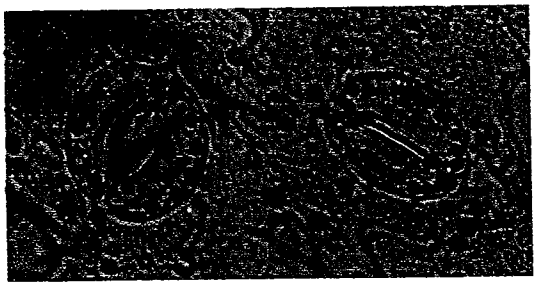

Figure 5. Stomata That Open (A), and Stomata Its closes (B) Tobacco Plant Varieties Srumpung On Stress *Waterlogging* Treatment

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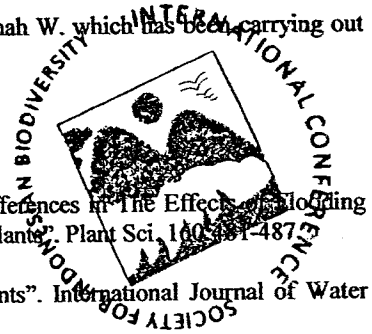

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Morpho-Physiological Analysis Of Some Tobacco Varieties (*Nicotiana Tabacum* L.) To Waterlogging Stress

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Abstract

Tobacco is considered as high-value crop that is sensitive to waterlogging stress. Several varieties has been commonly cultivated in Indonesia are Jepon Mawar, Jepon Banyak, and Rejeb. To date, little information has been reported about tobacco response to waterlogging stress. This research aims to determine response of some tobacco (*Nicotiana tabacum* L.) varieties to waterlogging stress. Some parameters measured were morphology and physiology. The result of this study showed Rejeb is positively response to waterlogging stress in term of its morfological aspect, including height of plant, width of leaf, number of adventitious roots and length of root, compared to other varieties. Waterlogging stress decreased the number of leaves in all varieties. In addition, chlorophyll content of all treated varieties has decreased, where the lowest value is response in Jepon Mawar (6.5 mg/L).

Key words: physiology, waterlogging, morphology, *Nicotiana tabacum* L.

Introduction

Tobacco is one of the main plantation commodities in East Java that have an important role in regional economic development through the provision of employment East Java Province, 2011) excise and tax (Kurniawan et al., 2014). The success of tobacco cultivation is strongly influenced by climate conditions (Nur and Apriana, 2013). The current condition with increased rainfall causes groundwater content to increase exceeding 85% in paddy fields and whereas on firmlands approximately less than 45%. Increased levels of high groundwater can decrease the quality of leaves, especially during the cooking period (Clough and Milthorpe, 1975). At high groundwater levels above 75% soil temperature becomes stable and high air humidity causes the resulting tobacco leaves to decrease (Belder et al., 2004). In tropical areas with high rainfall, there are often temporary or long standing inundations (Susilawati et al., 2012).

Waterlogging stress are abiotic stress that can affect growth and yield. The main cause of damage to the plant during the inundation is the lack of oxygen, so that the plants show wilting symptoms due to the absorption of nutrients and water are inhibited (Sairam et al., 2008). Waterlogging may also cause hypoxia and anoxic conditions in the soil, due to the low oxygen diffusion rate in water (Barrett, 2003). This low oxygen level can inhibit root respiration leading to changes in the respiratory path from aerobic to anaerobic (fermentation) (Voesenek et al., 2004).

Plants in anxious state will seek to survive by adapting mechanisms through adaptation of morphology, anatomy, physiology and metabolic mechanisms that allow plants to avoid and or tolerate stress factors both biotic and abiotic (Anton et al., 2002; Pourabdal in Susilawati et al ., 2012). The puddle-covered tobacco plants will increase the production of ethylene and Absisat Acid (Hurng, 1993). This increased ethylene hormone functions in the formation of an adventitious root that allows the roots to absorb oxygen from the air (Suwarti et al., 2013), whereas the increase in abscisic acid hormone will

cause early stomata closure (Parent et al., 2008). In addition, ethylene will induce cell death in the root cortex, which further forms the aerenchyma at the root. Development of aerenkim network is a form of adaptation of anatomy in conditions of inundation stress (Taiz and Zeiger, 2010). The morphological and anatomic response is a crop effort to survive.

Methodology

The study was conducted from June 2015 to August 2015 at Green House PT. SADHANA Pasuruan and Laboratory of Plant Bioscience and Technology FMIPA ITS. The research phase begins with the preparation of 3 varieties of tobacco varieties namely Rejeb, Jepon Mawar, and Jepon Banyak variety. Planting medium used is charcoal husk and compost with ratio 1: 2. The treatment of waterlogging stress was conducted on the 21st day after planting (HST). The waterlogging stress concentrations used are 100%, 150%, 175% and 200% of the field capacity. Harvesting of plants is done after 10 days of waterlogging stress. Planting medium used compost and charcoal soil with a ratio of 2: 1. Planting medium used 1.5 Kg / polybag. Furthermore, added NPK and SP-36 fertilizer as much as 5 grams per polybag. Field capacity measurements aimed at determining the watering volume used as the basis for flooding. Field capacity is calculated by the formula:

$$W = \frac{(Tb - Tk)}{Tk} x100\%$$

Information :
W = Field Capacity
Tb= Wet Weight
Tk= Dry weight

Seeds were added to planting medium consisting of coco peat and compost with a ratio of 1: 1. Germination done for 3 weeks 4 days. Furthermore, planting medium added NPK fertilizer (5 gr / lt media). Seeds that have reached the age of 25 Days After spread (HSS) moved into pottray containing 120 planting holes. Seeds are moved into pottray is a seed that has met the criteria, namely the emergence of 3-4 leaves. Seedlings grown for 15 days in pottray to then be planted on polybags. 40 Day After Sprinkling (HSS) seeds are transferred into polybags. Planting media in polybags consists of compost soil and charcoal husk with a ratio of 2: 1. Further fertilization done with NPK and SP-36 fertilizer as much as 5 gr / polybag. Provision of inundation stress for 10 days. Treatment was performed at the time of the plant at 21 HST Staking congestion used 100%, 150%, 175% in and 200% above field capacity.

Figure 1. The Treatment of Waterlogging Stress Cutting on Many Jepon Variety Tobacco Plant: (A). 100%, (B). 150%, (C). 175%, (D). 200%.

Results and Discussion

Plant height

The first response was shown on the Jepon Mawar and Rejeb varieties which relative to the increase in plant height after flooding as in Table 1. The ability of plant height increases depends on the genetic characteristics of the variety and is influenced by the environment or level of plant development prior to waterlogging stress (Jackson and Ram, 2003; Kawono et Al., 2009). While the opposite response is indicated by Jepon Many varieties which tend to decrease plant height along with increasing waterlogging stress level.

Table 1. Observation Result of Plant Growth Rate of Several Varieties of Tobacco after Treatment of Waterlogging

Varieties	Plant High Growth Rate ± STD (cm) in Waterlogging stress Presentation			
	100%	150%	175%	200%
Rejeb	18,8 ± 1,0 bcde	17,1 ± 2,7 def	26,9 ± 1,7 a	23,5 ± 3,0 ab
Jepon Mawar	12,9 ± 0,6 f	14,6 ± 2,0 ef	14,0 ± 5,2 ef	18,4 ± 2,4 cde
Jepon Banyak	22,3 ± 0,6 abc	20,4 ± 1,8 bcd	20,2 ± 0,6 bcd	19,6 ± 0,5 bcd

Description: The mean followed by the same letter does not show any significant difference with the Tukey test (p = 0.05). The number behind the ± sign is the Deviation Standard (STD).

The response to the growth of the stagnant plants was mediated by at least 3 different hormones, Giberelin Acid (GA), Absisic Acid (ABA) and ethylene (Kende et al., 1998); (Kawono et al., 2002). These three hormones play a role in shoot extension (Voesenek et al., 2003). Ethylene plays an important role in responding to waterlogging stress through the regulation of Giberelin and ABA, which is a positive and negative regulator in shoot elongation (Saika et al., 2007). The accumulation of ethylene when the plant is waterlogging stress will regulate the decrease in ABA content. This decrease in endogenous ABA content in this internode is needed to stimulate the expression of (GA) 3-oxidase, the enzyme used to catalyze it into the bioactive form of Giberelin (GA1) so that the GA concentration increases (Salazar et al., 2015). One of GA's responses is in the process of displaying stems. GA enhances the shifting of carbohydrates in shoots to produce sugars as energy for proton activity and cell wall formation (Voesenek et al., 2003). So it can be assumed that Rejeb and Jepon Mawar varieties have decreased ABA concentration when the waterlogging stress is used to stimulate the increase of GA concentration.

Leaf Area

The result of Anova test is continued with Tukey test which gives the result that the waterlogging stress treatment factor significantly affect the leaf area of the three varieties shown in Table 2.

Table 2. Observations Mean Size Tobacco Leaves Several Varieties after Treatment of Waterlogging Stress

Varieties	Average Growth Area of Leaves ± STD (cm2) on Waterlogging Stress Presentation			
	100%	150%	175%	200%
Rejeb	83,6 ± 5,0 bc	77,2 ± 17,0 c	137,6 ± 28,5 a	108,8 ± 18,2 ab
Jepon Mawar	54,9 ± 18,9 c	66,6 ± 15,9 c	65,5 ± 4,2 c	123,0 ± 14,2 a
Jepon Banyak	122,7 ± 8,8 a	83,8 ± 2,6 bc	81,7 ± 1,5 bc	81,4 ± 0,8 bc

Description: The mean followed by the same letter does not show any significant difference with the Tukey test (p = 0.05). The number behind the ± sign is the Deviation Standard (STD).

Based on Table 1 it can be seen that the leaf area of tobacco plants in waterlogging stress has decreased in Rejeb varieties treated 150% above the field capacity. Whereas in Jepon Banyak variety, leaf area of tobacco plants decreased along with increasing of flooding level. Decreased leaf area is one of the symptoms of plant growth decline that can be observed when the plants experience hypoxia or anoxia. According to Sena and Kozlowski (1998), at the time of hypoxia or anoxic conditions the plant will show a decrease in chlorophyll synthesis due to the accumulation of ethylene and / or decreased cytokinin synthesis. Ethylene is one of the hormone groups in the form of hydrocarbon gas which has a significant effect on the development of shoots and roots. Increased ethylene synthesis is one form of crop response to stress and is widely produced by aging or maturation tissue (Hopkins and Huner, 2009).

Root Length

The result of Anova test is continued with Tukey test which gives the result that the waterlogging treatment factor significantly affect the root length of the three varieties shown in Table 3.

Table 3. Observation Result of Root Length of Several Varieties of Tobacco after the Treatment of waterlogging Stress

Varieties	Average Root Length ± STD (cm2) on Waterlogging Stress Presentation			
	100%	150%	175%	200%
Rejeb	17 ± 4,9 abc	15 ± 5,7 abc	19,7 ± 6,9 ab	19,4 ± 4,7 abc
Jepon Mawar	14,6 ± 5,2 abc	11,5 ± 1,6 abc	11 ± 4,0 abc	12,9 ± 4,8 abc
Jepon Banyak	19,9 ± 6,7 a	11,3 ± 0,7 abc	10,1 ± 0,9 bc	9,8 ± 1,3 c

Description: The mean followed by the same letter does not show any significant difference with the Tukey test (p = 0.05). The number behind the ± sign is the Deviation Standard (STD).

Based on Table 3 shows that the root length of Rejeb varieties is relatively increasing in root length. Rejeb varieties have an increasing number of leaves as the percentage of waterlogging stress increases. This increase in the number of leaves indicates that water and nutrients can be optimally distributed to the canopy portion of the plant. This may indicate that the root area of the rejected variety of Rejeb suffers little damage. This can be supported by the high number of adventitious roots in this variety when it is waterlogging stress. While in Jepon varieties Many treatments of 150%, 175% and

200% above the field capacity had a lower root length value when compared with the treatment 100% above the field capacity. This shows the inhibition of root growth with increasing percentage of standing water. According to Hapsari and Adi (2010), in stagnant conditions, the soil pore volume containing air less than 10% thus inhibits root growth. Lack of oxygen in soil due to waterlogging stress is a limiting factor of plant growth and productivity.

Inhibition of root growth and elongation may also be due to differences in suberin deposition of each tobacco varieties on the cell wall of the cortex or root eksodermis involved in the ROL (Radical Oxygen Loss) mechanism. In connection with this, allegedly Rejeb varieties have more levels of suberin content than other varieties. According to Visser et al., (2000), the loss of oxygen from the roots depends on the presence of a barrier that can block the radial oxygen loss to the surrounding soil. Some plant species that live in fertile (wet) areas, have a barrier on the outside of the root cortex that prevents oxygen loss from the roots (Armstrong, 2005). In the presence of such a barrier, oxygen diffuses longitudinally toward the root tip so as to increase aeration at the root tip (forming aerobic zone) and allow root elongation on the stagnant soil (Pedersen et al., 2004). The aerobic zone formed can also prevent the accumulation of toxic compounds such as Fe2 +, Mn2 +, and sulphides around the root tip (Soukup et al., 2002).

The Formation of Adventif Roots

Adventitious roots are roots that grow at the base of the stem or lenticel-rich plant parts, growing laterally parallel to the water or soil surface (Parent et al., 2008). Adventitious roots can appear naturally from the stem tissues under conditions of environmental stress; This root can also be induced because of mechanical damage or regeneration of shoot tissue cultures (Li et al., 2009). This root is a postembryonic root that appears on stems and leaves and from non-pericidal tissue in old roots (Geiss et al., 2009).

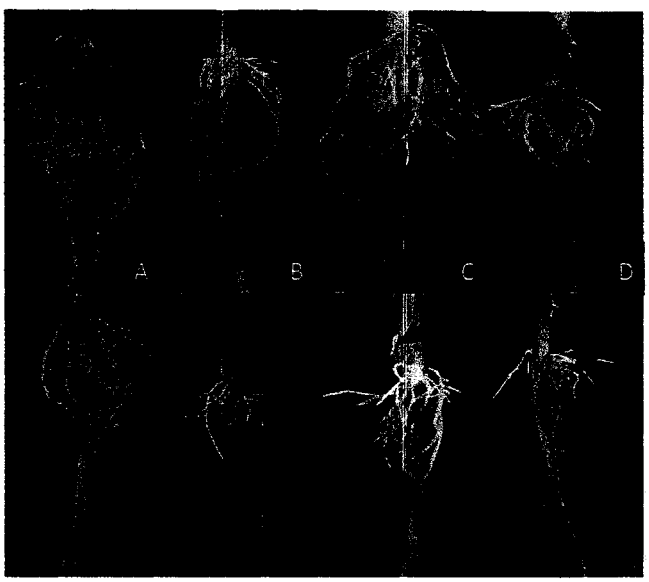

Figure 2. Adventitious Root Formation in Third Tobacco Plant Varieties of Test after 10 Days of Waterlogging Stress Treatment.

The formation of adventif and aerenchyma roots is an indicator of the mechanism of plant adaptation in most tolerant waterlogging stress plants (Akhtar and Nazir, 2013). Adventitious roots are roots arising from stems, or other parts of plants (such as leaves in Sedum and Begonia plants), and areas of the main roots other than the proximal bearing into the elongation zone (Beck, 2010). Adventitious roots develop from primordial roots, which can appear in many tissues in the plant stem.

As with lateral roots, these primordia differentiate into root cell types, and vascular tissue is formed in the presence of xylem and phloem in the original roots (Pugnaire & Valladares, 2007).
The presence of adventitious roots helps the absorption of water and nutrients in plants that are tolerant to waterlogging stress. Adventitious roots also facilitate the final product of alcohol fermentation, ethanol to diffuse from plants, so as not to accumulate in plants. According to Smith et al. (2010), some plants produce adventitious roots in response to oxygen loss. These additional roots replace damaged roots and are more effective in oxygen transfers. This root has a more effective function due to the presence of aerenchyme tissue in it and grows in the surface area of the soil which generally oxygen levels are still high. The formation of aerenchyme shows that the plant is inundated with a response to the condition of hypoxia and anoxia. Increased porosity of aerenchyma may increase the exchange toward the root of the plant and improve the aeration process in roots that are in the waterlogging stress (Parent et al., 2008).

Conclusion

Waterlogging stress can provide a different response in each tobacco plant varieties. In morphological parameters (plant height and leaf area), Rejeb varieties and Jepon Mawar varieties have different responses to the varieties of Jepon Banyak. Rejeb and Jepon Mawar varieties are relatively increasing plant height and leaf area. While on root length parameter, Jepon Mawar and Jepon Banyak varieties have decreased root length along with increasing inundation rate. The four test varieties showed equal response of adventif root formation at 150%, 175% and 200% treatment which was general morphological response when gripped waterlogging stress.

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4th IBOC 2018

PEMANFAATAN TANAMAN TEMBAKAU ...

HERY PURNOBASKORO

Profile of Protein Levels And Cholorophyl Content Some Tobacco Varieties(*Nicotiana tabacum* L.) On Waterlogging Stress

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Abstract

Tobacco is a high-value crops that are sensitive to waterlogging stress. Some tobacco varieties have been widely cultivated in Indonesia, including Jepon Mawar, Jepon Banyak and Rejeb. Some species have different abilities to withstand the conditions of waterlogging stress by morphological adaptation, anatomy, physiology, and metabolic pathway changes. Changes in protein profiles is one form of plant defense response to waterlogging stress. Profile proteins experienced upregulation in hypoxia and anoxia conditions when gripped waterlogging stress known as anaerobic polypeptides. In addition to the waterlogging stress will cause the decrease in chlorophyll levels as a result of chlorosis during the stress. The purpose of this research is to know protein profile and chlorophyll content of several varieties of tobacco (Nicotiana tabacum L.): Rejeb, Jepon Mawar and Jepon banyak against stagnant waterlogging stress. The protein profile was analyzed by SDS-PAGE method. While the chlorophyll content was analyzed by spectrophotometric method. The protein profiles expressed from the three test varieties were present in the molecular weight range 85.38-153.33 kDa. Proteins with a molecular weight of 85.38 kDa are thought to have similarities to the Peroxsidase group (BM = 85 kDa) that play a role in the ROS detoxification process. While the chlorophyll content in the three varieties decreased with the increasing of waterlogging stress except on Rejeb varieties treated by 175% and Jepon Mawar varieties on 200% waterlogging stress treatment.

Keywords: Waterlogging stress , *Nicotiana tabacum* L., protein profile and chlorophill

1. INTRODUCTION

Waterlogging of an abiotic stresses which may affect growth and yields. One of the causes of waterlogging is the high rain insensity. The main cause of damage to the plant during the waterlogging is the low oxygen, which indicated the existence of wilting symptoms due to the absorption of nutrients and water that hampered [1]. Waterlogging can also cause the condition of hypoxia and anoxia in the soil. Stress reduces the pool of gas exchange between the plant tissue and air as gas diffusion layer in water 10,000 times slower than in air [4]. Despite the low oxygen availability is an important factor affecting plant growth, soil chemical element content, such as pH and redox potential, is also changing under waterlogging stress conditions and can also affect the survival and growth of plants [5,6

Lack of oxygen stimulates anaerobic fermentation that affect less well on some morphological and physiological processes, such as photosynthesis, energy metabolism, redox potential, gene expression, as well as degradation and protein synthesis [7]. One form of plant responses to abiotic stress is the change in protein expression and post-translational modification of proteins to activate the defense system in the face of stress [8]. The protein profile changes in hypoxia. In addition, also expressed specific proteins that have upregulation in this case is anaerobic polypeptides. The enzyme plays a role in the metabolism of glucose, glycolysis, fermentation [9], hormone synthesis, programmed cell death [10].

Lack of oxygen stimulates anaerobic fermentation that affect less well on some morphological and physiological processes, such as photosynthesis, energy metabolism, redox potential, gene expression, as well as degradation and protein synthesis [7]. One form of plant responses to abiotic stress is the change in protein expression and post-translational modification of proteins to activate the defense system in the face of stress [8]. The protein profile changes in hypoxia. In addition, also expressed specific proteins that have upregulation in this case is anaerobic polypeptides. The enzyme plays a role in the metabolism of glucose, glycolysis, fermentation [9], hormone synthesis, programmed cell death [10]. Reported by Ahzan (2007), found a total of 35 proteins in the roots of tomato plants (*Lycopersicum esculentum*) in response to the waterlogging stress 16 proteins had increased expression and 13 proteins had decreased expression. In maize (*Zea mays*), the treatment of waterlogging stress changes the pattern of protein synthesis in the primary root [12].

Response of the other plant in response to the waterlogging stress is a decrease in the conductance of stomata (Folzer et al., 2006), changes in hormone balance (Else et al., 2001), decreased transpiration and inhibition of photosynthesis rate due to a decrease in leaf chlorophyll content (Cao and Conner, 1999). These responses occur within hours or days, depending on the level of tolerance of plant species (Striker et al., 2005). The decline of chlorophyll and chlorophyll degradation massively occur during leaf senescence, fruit ripening, and also in response to environmental stress (Hortensteiner and Bernhard, 2010). The decline in the chlorophyll content due to intake of nutrients, especially of N low due to damage to the root system due to flooding.

Determination of protein profiles and chlorophyll content of some varieties of tobacco in response to waterlogging stress provide a better understanding of its function in adaptation to stress.

2. RESEARCH METHOD

2.1 Preparation of tobacco seeds

Tobacco varieties seed in this study include var. Rejeb, Jepon Mawar, and Jepon Banyak. The seeds were germinated in germination media containing compost and chaff (2:1). Germination was carried out for 15 days after seeding (15 das). Germinated seeds were then pricked (until seedlings aged 54 das) and transferred *pottray*.

2.2. Planting tobacco seeds in polybag

Tobacco seedlings 54 das were transferred to non-perforated polybags. Planting medium used in this procedure was compost and chaff with a ratio of 4:1. Each polybag contains 1 kg of planting medium. 5 gr NPK fertilizer was applied for each plant. Pest and disease control was done using insecticides organtrin 1.5 ml / L. The control carried out at the time of old tobacco plants (15 dap). Tobacco plants were grown for 3 weeks (21 days after planting (dap).

2.3. Field capacity measurement

Measurements of field capacity were conducted to determine the volume of the water in the treatment of *waterlogging stress*. Planting media that were placed in polybag were watered until the water passed through the media. The media was subsequently left to stand for 3 days until there were no the dripping water occurred. Furthermore, the media were directly weighted. Meanwhile, dry weight media were measured by placing the planting medium in the oven at 100° C for 24 hours until reaching a constant weight. Field capacity is calculated using the formula:

$$W = \frac{Tb - Tk}{Tk} \times 100\ \%$$

Information:
W : Field Capacity
Tb : Wed Weight (Gram)
Tk : Dry Weight (Gram)

2.4. Stress Waterlogging Treatment

Waterlogging stress treatments were carried out using 21 dap tobacco plants. *Waterlogging stress* treatment used in this study were 100%, 150%, 175% and 200%. These treatments were applied during 10 days. The volume of water was maintained for 10 days of treatment.

2.5 Sample Preparation (Protein Extraction)

Protein profile analysis using protein electrophoresis method. Plants that have been taken are washed with distilled water. A total of 0.250 grams of leaf organs were washed with phosphate buffered saline (PBS) pH 7.4. The organs were homogenized with cold mortar and added 500 µl of protein extract buffer. Homogenate is inserted in a 1.5 ml tube and centrifuged at 10000 rpm at 4 ° C for 10 minutes. The pellet is removed and the supernatant is inserted a new 1.5 ml tube then stored at -20 °C. For electrophoresis used 12.5% separating gel and 5% stacking gel.

2.6 Sample Preparation (Chloropyl Content)

Leaf chlorophyll was measured by spectrophotometric method using the modified Hall and Rao method in Priadi (2014). Leaves weighing 0.1 grams were crushed in porcelain porcelain and dissolved in 10 mL of 95%

methanol for 24 hours in the dark. Furthermore, the extract was filtered with Whatman no. 1 and The solution is introduced into the test tube and covered with aluminum foil. Then the 3 ml solution was introduced into the cuvette and measured its absorbance by spectrophotometer at 645 and 663 nm wavelengths. Chlorophyll levels can be expressed in mg of chlorophyll per gram of material (Yoshida et al., 1976 in Rachmawati and Retnaningrum, 2013)). Total chlorophyll content is calculated by the formula (Harborne, 1987):

2.7 Parameters of observation

Parameters include the observation of profile protein and content chlorophyll.

2.8 Data Analysis

The data chlorophyll content obtained were analyzed using ANOVA Two Way followed by *Tukey* test and descriptive to analysis provile protein.

3. RESULTS AND ANALYSIS

3.1 Protein Profile Jepon banyak Variety

Based on the results of SDS-PAGE analysis Jepon banyak varieties are shown in Figure 1 shows that a protein with a molecular weight of 153.33 kDa decreased expression represented by the thickness of the protein band with increasing level of flooding. Protein band with a molecular weight of 119.51 and 103.98 kDa expressed only in the treatment of 100% waterlogging stress and degradation as indicated by the loss of protein bands on the treatment of 150%, 175% and 200%. The protein band with a molecular weight of 90.48 kDa is expressed only in the concentration of 175% and 200% waterlogging. While the protein with a molecular weight of 85.38 kDa was uniformly expressed on all the treatment of waterlogging stress. The results show that there are some proteins that degrade or decrease expression when the waterlogging stress. In addition there are also proteins that are only expressed at high waterlogging rates such as proteins with molecular weight of 90.48 kDa. The weight of expressed protein molecules differs across all treatments ranging from 85.38-153.33 kDa.

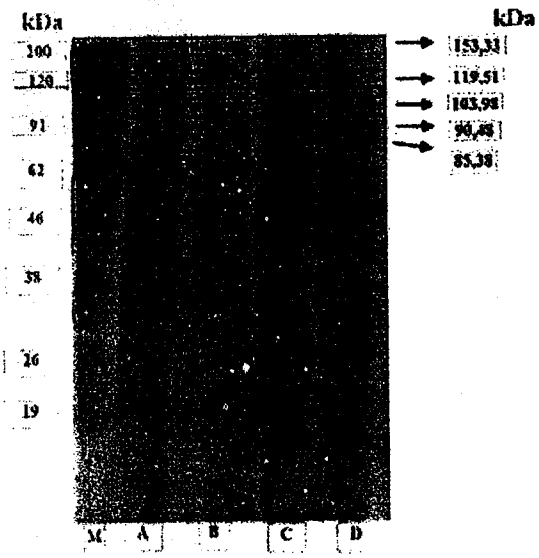

Figure 1. Results of Analysis of SDS-PAGE Profiles of Jepon Banyak Variety Proteins. : (M) Marker; (B) 100% Waterlogging stress above the field capacity; (C) 150% Waterlogging stress above the field capacity (D) 175% Waterlogging stress above the field capacity (E) 200% Waterlogging stress above the field capacity.

Research on tomato plants (*Solanum lycopersicum*) treated with waterlogging for 14 days showed that expressed proteins ranged in molecular weight 50-110 kDa [21]. The 50-55 kDa molecular weight protein is a member of the Rubisco Large-Subunit (RLS). The 60-61 kDa molecular weight protein is Rubisco Binding Protein (RBP), a molecular weight protein of 93-95 kDa which is an ATP-dependent protease belonging to serine protease (Clp-P), and a protein with a molecular weight of 110 kDa is a protein Rubisco Activase (RA). Based on the results of identification, these proteins play a role in the process of photosynthesis. Rubisco's expression decreased in total dissolved protein during response to the waterlogging stress. Rubisco has two functions that act as carboxylases that mediate CO2 assimilation and as oxygenase in catalyzing the early photorespiration stage [22]. In addition, the presence of waterlogging stress can also induce the formation of ROS (Reactive Oxygen Species) which may lead to the degradation of Rubisco subunit and Rubisco Activase.

3.2 Protein Profile Rejeb Variety

In Rejeb varieties, occurring protein with molecular weight of 153.33 kDa increased expression in the treatment of waterlogging stress 175% as indicated by the band thicker than other treatments. Protein with a molecular weight of 119.21 and 103.98 kDa was also expressed on the previous varieties. These proteins are expressed in the treatment of 100% and have been degraded in the treatment of 150%, 175%, 200%. In addition, there is an increased expression of the protein bands with molecular weight of 85.38 kDa when the waterlogging stress treatment 175%, it is seen from the thickness of the protein bands. Band thickness decreases as 200% waterlogging stress concentration as shown in Figure 2.

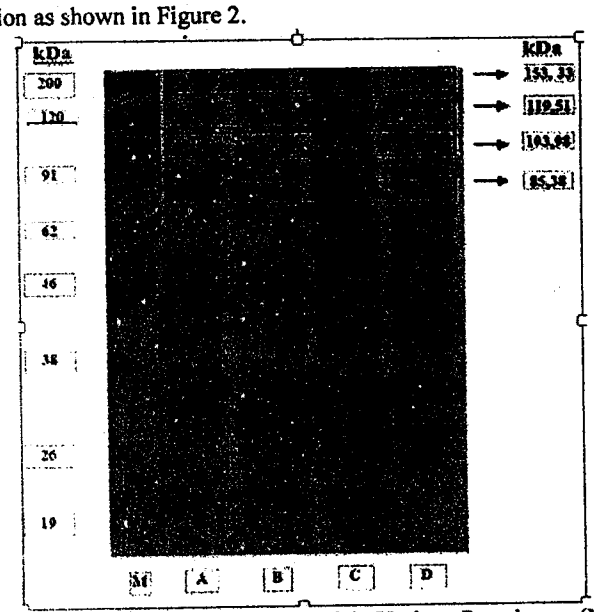

Figure 2. Results of Analysis of SDS-PAGE Profiles of Rejeb Variety Proteins. : (M) Marker; (B) 100% Waterlogging stress above the field capacity; (C) 150% Waterlogging stress above the field capacity (D) 175% Waterlogging stress above the field capacity (E) 200% Waterlogging stress above the field capacity.

According to [23], maize (*Zea mays*) were flooded for 52 hours showed increased expression of a protein with a molecular weight of 85 kDa and 133 kDa. Protein with a molecular weight of 133 kDa initially decreased expression during the initial 4 hours of waterlogging stress, and increased after 28 to 52 hours of waterlogging stress. While the protein with a molecular weight of 85 kDa significantly increased its expression during 52 hours of waterlogging stress. Based on the results of identification, both proteins are included in the peroxidase group that play a role in the physiological functions of plants including plant development, oxidative stress and processes associated with plant cell walls. Peroxsidase also plays a role in the production and detoxification of ROS (Reactive Oxygen species) caused by stress.

Oxidative stress occurs when unbalanced production and neutralization of ROS within cells. High light, heat, pathogen attack, low oxygen levels, and re-aeration after hypoxia phase can increase ROS production [25]. ROS is produced from molecular oxygen through several reduction steps. Anion superoxide (O_2-), hydroxyl radical (OH), singlet oxygen ($^1O_2$) results from the reduction of one or three electrons from oxygen by the reduction energy provided by the electron carriers in mitochondria and chloroplasts [26]. ROS is highly reactive and can cause damage to lipid membranes and proteins [23]. Plants have a protective system to protect mitochondria from overproduction of ROS such as antioxidants (glutathione, ascorbic acid, tocopherol, tannins, ubiquinol, and phenolic acid), ROS scavenging enzymes such as superoxide dismustase (SOD), catalase (CAT), ascorbate peroxidase (APX), and gluthatione peroxidase (GPX). So it can be predicted that an increase in protein expression with a molecular weight of 85.38 kDa in the Rejeb variety (175% stroke) is one form of protection mechanism of oxidative stress that can damage the lipid membrane and other proteins. It is also supported with Rejeb reef morphology data of 175% treatment which showed better growth compared to 100% treatment.

3.3 Protein Profile Jepon Mawar Variety

In Jepon Mawar varieties, also express proteins that appeared in previous varieties as shown in Figure 3, which is a protein with a molecular weight of 119.51 and 103.98 kDa that appears only in the treatment of

waterlogging stress 100%. Protein with a molecular weight of 153.33 kDa stress treatment increased expression as a pool of 200%.

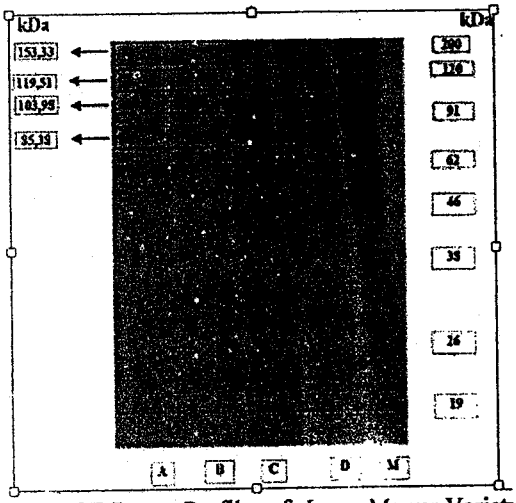

Figure 3. Results of Analysis of SDS-PAGE Proten Profiles of Jepon Mawar Variety : (M) Marker; (B) 100% Waterlogging stress above the field capacity; (C) 150% Waterlogging stress above the field capacity (D) 175% Waterlogging stress above the field capacity (E) 200% Waterlogging stress above the field capacity.

Increased expression also occurs in Jepon Mawar varieties with 175% stress treatment. While the protein with a molecular weight of 85.38 kDa expressed only in the treatment of a pool of 100% stress and degradation or decreased expression with increasing level of flooding. So it can be said that the majority of proteins that appear on the treatment of 100% have been degraded in the other treatment.

4. Chlorophyll Content

The negative impact of the waterlogging stress is inhibition of leaf growth, biomass reduction, a decrease in stomatal conductance and decrease the amount of chlorophyll a and b in the leaves [4]. The decrease in chlorophyll occurs significantly in older leaves, indicating rapid degradation of chlorophyll in leaves near the stagnant roots. Their stress puddle on 3 different varieties show varying levels of chlorophyll content. This is shown in Figure 4.

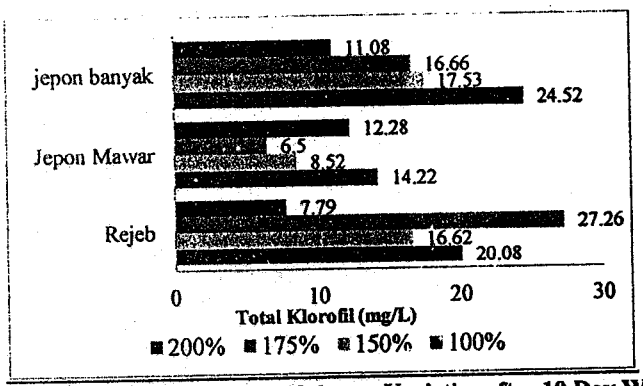

Figure 4. Average Total Chlorophyll Several Tobacco Varieties after 10 Day Waterlogging Stress

According to Fig. 4, it can be seen that the total chlorophyll in the three varieties decreased except in Rejeb varieties treated by waterlogging stress 175% and Jepon varieties on 200% waterlogging stress treatment. The decrease in chlorophyll levels is thought to be caused by the destruction of stagnant roots. Waterlogging stress can cause the death of cells that can inhibit root development and function of the root system such as fetching water and nutrients, especially nitrogen, so that the need for water and N of the canopy is not met. As a result leaves wither and yellow. One of the benefits of N to the plants is for the formation of chlorophyll in the leaves. Chlorophyll degradation can be demonstrated through chlorosis on the leaves that gripped puddle (Pociecha et al., 2008) as shown in Figure 4.

4. CONCLUSION

Stress can alter protein expression inundation of some varieties of tobacco. SDS-PAGE analysis of tobacco leaf protein indicates that there are 5 different proteins expressed in each variety and treatment of waterlogging stress. proteins are expressed differently in all three test varieties contained in the molecular weight range from 85.38 to 153.33 kDa. Varieties Rejeb increase protein expression at a molecular weight of 85.38 kDa in the treatment of 175%, as indicated by the thickness of the protein bands. The protein is degraded in the varieties of roses Jepon treatment 150%, 175% and 200% and expressed the same in all treatment Jepon Many varieties. Protein with a molecular weight of 85.38 kDa allegedly has in common with Peroxsidase group (molecular weight 85 kDa), which plays a role in detoxification of ROS.The chlorophyll content in the three varieties decreased with the increasing of waterlogging stress except on Rejeb varieties treated by 175% and Jepon Mawar varieties on 200% waterlogging stress treatment.

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