LAPORAN AKHIR
Penelitian Unggulan Perguruan Tinggi (U)

ngembangan Bibit Jeruk dengan Masa Juvenil Singkat Secara *In Vitro* dan Perbanyakan Masal Menggunakan Sistem Kultur Bioreaktor

TIM PENGUSUL
Dr. Dra. Wahyu Widoretno, M.Si. NIDN 0014046307
Nanang Suryadi, SE, MM. NIDN 0008077305
Dr. Serafinah Indriyani, M.Si. NIDN 0009096306
Chaireni Martasari, SP, M.Si. NIDN -

Dibiayai oleh:
Direktorat Jendral Pendidikan Tinggi,
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Judul Penelitian	: Pengembangan bibit jeruk dengan masa juvenil singkat secara *in vitro* dan perbanyakan masal menggunakan sistem kultur bioreaktor
Peneliti/Pelaksana	
Nama Lengkap	: Dr. Dra. Wahyu Widoretno, M.Si.
NIDN	: 0014046307
Jabatan Fungsional	: Lektor
Program Studi	: Biologi, Fakultas MIPA
No HP	: 085649766038
Alamat surel (e-mail)	: wahyu_widoretno@yahoo.com
Anggota 1	
Nama lengkap	: Nanang Suryadi, SE. ME
NIDN	: 0008077305
Perguruan Tinggi	: FEB Universitas Brawijaya
Anggota 2	
Nama lengkap	: Dr. Serafinah Indriyani, M.Si.
NIDN	: 0009096306
Perguruan Tinggi	: FMIPA Universitas Brawijaya
Anggota 3	
Nama lengkap	: Chaereni Martasari, SP
NIP	: 19710308 200212 2 001
Instansi	: Balitjestro
Institusi Mitra	: -
Tahun Pelaksanaan	: Tahun ke 1 dari rencana 3 tahun
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Malang, 15 November 2013

Mengetahui,
Dekan

Prof. Dr. Marjono, M.Phil.
NIP. 19621116 198803 1 004

Ketua,

Dr. Dra. Wahyu Widoretno, M.Si.
NIP. 19630414 198903 2 001

Menyetujui,
Ketua LPPM UB

Prof. Dr. Ir. Siti Chuzaemi, MS
NIP. 19530514 198002 2 001

ABSTRAK

Kebutuhan buah jeruk di Indonesia terus meningkat sementara produksi menurun dari tahun ke tahun. Untuk memenuhi kebutuhan konsumsi jeruk nasional dan bahan mentah industri maka diperlukan pengembangan budidaya yang memadai, salah satu prasyarat untuk itu adalah ketersediaan bibit dalam jumlah banyak dan seragam. Perbanyakan tanaman jeruk secara *in vitro* melalui embriogenesis somatik mempunyai masa juvenil panjang sehingga menjadi kendala dalam pengembangan tanaman jeruk. Sehingga perlu upaya untuk mengembangkan teknologi perbanyakan in vitro batang atas jeruk dengan masa juvenile singkat. Penelitian selama 3 tahun ini bertujuan untuk mengembangkan teknologi perbanyakan jeruk batang atas dengan masa juvenil singkat, teknologi perbanyakan bibit jeruk masal dengan menggunakan sistem kultur bioreaktor dan bibit jeruk tanpa atau dengan masa juvenil singkat dan usaha penyedia bibit jeruk unggul serta industri penyedia bibit jeruk. Pada tahun pertama kegiatan penelitan yang dilakukan meliputi pematahan juvenilitas embrio somatik dengan modifikasi medium dan kondisi kultur, maturasi dan regenerasi embrio somatik hasil perlakuan, minigrafting plantlet dengan batang bawah jeruk JC, evaluasi juvenilitas tanaman hasil minigrafting, analisis ketahanan penyakit dengan uji ELISA dan analisis indentitas true-to-type regeneran dengan penanda molekuler SSR. Dari hasil penelitian ini telah diperoleh metode untuk pematahan juvenilitas tanaman regeneran dengan perlakuan modifikasi medium dan kondisi kultur untuk pertumbuhan embrio somatik dan metode maturasi embrio somatik jeruk. Plantlet hasil regenerasi embrio somatik tersebut juga telah digraftingkan dengan batang bawah jeruk JC. Sebagian tanaman minigrafting menunjukkan masa juvenil singkat, bebas virus CTV dan true-to-type. Metode ini pada tahun kedua penelitian dijadikan dasar untuk melakukan kajian scaling up produksi bibit menggunakan sistem kultur bioreaktor. Embrio somatik hasil perlakuan yang menghasilkan tanaman mempunyai potensi tingkat juvenile singkat, bebas penyakit dan true-to-type ini akan diperbanyak secara masal menggunakan sistem kultur bioreaktor.

Penggunaan bioreaktor dengan medium cair untuk perbanyakan tanaman sangat menguntungkan karena kemudahan *scaling-up*, kemampuan untuk mencegah gangguan fisiologi pucuk dan hiperhidrisitas daun dan biaya produksi rendah. Perbanyakan skala besar menggunakan bioreaktor juga bermanfaat karena produksi propagul sepanjang tahun, biaya murah dan waktu lebih singkat. Sehingga dengan didapatkannya metode perbanyakan masal bibit dengan sistem bioreaktor ini diharapkan akan diperoleh bibit jeruk dengan masa juvenile singkat secara masal dan kontinyu. Sehingga pada tahun ketiga penelitian telah dapat disediakan bibit jeruk yang siap dipasarkan pada kelompok petani dan penangkar bibit serta menjalankan usaha penyediaan bibit dalam skala besar.

ABSTRACT

Citrus fruit demand in Indonesia is increasing continuously while the production is declining from year to year. In order to provide for fulfillment of national consumption and of industrial raw materials, it needs developing an appropriate cultivation, one of those is the availability of quality seed in large quantities and uniform. In vitro propagation of citrus was carried out using somatic embryogenesis, however the technique ensures only the propagation of citrus rootstock, since these plants derived from somatic embryogenesis has a long juvenile period. Therefore, to overcome the problems mentioned above, it needs to be developed in vitro propagation technology of citrus stem with short juvenile period. The research objectives for 3 years are to develop in vitro propagation technology of citrus stem with short juvenile period, to develop mass propagation technology of citrus seed by using bioreactor culture system, to obtain superior seed without or with short juvenile period, and to develop superior seed supplier as well as citrus seed supplier industry. In the first year of research were conducted: the development of methods to shorten the juvenile period in citrus resulted form somatic embryogenesis by modification medium and culture condition, maturation of treated somatic embryo and plant regeneration, grafting plantlet with Javanche Citroen (JC) root stock, evaluation juvenility level of regenerants, analysis of disease virus by using ELISA, and analysis of true-to-type regenerants by using SSR molecular marker. The result of this research has obtained the method to shorthen juvenility of plant regenerated from somatic embryo were cultured on modified medium and condition culture and methods of somatic embryo maturation and plant regeneration. Plantlets regenerated from the somatic embryo have grafted with Javanche Citroen (JC) rootstock. Some of minigrafting plants showed short juvenile period, Citrus Tristeza Virus free and true-to-type. This method will be used as the basis for studying of scaling up of seed production using bioreactor culture system in the second year research. Treated somatic embryo that produced plants has potency short jevenile period, virus free, and true-to-type will be propagated using mass bioreactor culture system. Plant propagation using bioreactor system should be an advantageous method in term of ease of scaling up, ability preventing physiological disturbance of shoot and leave hyperhydricity and low cost production. Large-scale micropropagation using bioreactor is also beneficial for seed production throughout the year, low cost and short time. Realizing mass propagation method of citrus seed by using bioreactor culture system will be obtained citrus seed, which have short juvenile period in large amount and continuously. Therefore in the third year of research, marketed citrus seed to farmer community can be provided and also seed supplier industry can be developed.

RINGKASAN

Kebutuhan buah jeruk di Indonesia terus meningkat dari tahun ke tahun. Untuk memenuhi kebutuhan konsumsi jeruk nasional dan bahan mentah industri maka diperlukan pengembangan budidaya yang memadai, salah satu prasyarat untuk itu adalah ketersediaan bibit dalam jumlah banyak dan seragam. Perbanyakan tanaman jeruk secara *in vitro* telah dilakukan melalui embriogenesis somatik, tetapi teknik ini hanya menghasilkan batang jeruk bawah karena tanaman yang berasal dari embriogenesis somatik ini mempunyai masa juvenil panjang sehingga menjadi kendala dalam pengembangan tanaman jeruk. Sehingga perlu upaya untuk mengembangkan teknologi perbanyakan in vitro batang atas jeruk dengan masa juvenile singkat.

Penelitian selama 3 tahun ini bertujuan untuk mengembangkan teknologi perbanyakan jeruk batang atas dengan masa juvenil singkat, teknologi perbanyakan bibit jeruk masal dengan menggunakan sistem kultur bioreaktor dan bibit jeruk tanpa atau dengan masa juvenil singkat dan usaha penyedia bibit jeruk unggul serta industri penyedia bibit jeruk.

Pengembangan teknologi perbanyakan jeruk batang atas dengan masa juvenil singkat dilakukan dengan memodifikasi kondisi kultur dengan suhu dingin dan fotoperiode dan media kultur dengan pemakaian giberelin dan sukrosa tinggi. Evaluasi tingkat juvenilitas dilakukan dengan mengevaluasi ada tidaknya duri sebagai penanda juvenilitas pada tanaman berumur 3 bulan setelah dilakukan minigrafting dengan jeruk batang bawah JC (Javanche Citroen). Identifikasi identitas genetik *true-to-type* dan bebas penyakit pada regeneran dilakukan dengan analisis molekuler dengan penanda SSR (*Simple Sequence repeat)* dan uji ELISA. Sedangkan perbanyakan masal bibit jeruk yang mempunyai masa juvenil singkat, bebas penyakit dan true-to-type dengan menggunakan sistem kultur bioreaktor. Dalam upaya untuk melakukan pemasaran bibit yang telah dihasilkan, akan dilakukan beberapa langkah kegiatan meliputi identifikasi penangkar bibit dan kelompok petani jeruk, merancang *marketing communication* untuk penangkar bibit dan kelompok petani yang menjadi target, sosialisasi dan kerjasama pemasaran dengan penangkar bibit dan kelompok petani jeruk.

Dari kegiatan penelitian yang dilakukan selama 3 tahun ini diharapkan akan diperoleh teknologi perbanyakan jeruk dengan masa juvenil singkat, *true-to-type* dan bebas penyakit, teknologi perbanyakan masal bibit dengan menggunakan sistem kultur bioreaktor, bibit jeruk batang atas dengan masa juvenil singkat dan industri penyedia bibit jeruk unggul, publikasi ilmiah dalam jurnal Nasional dan Internasional, paten terkait teknologi perbanyakan jeruk dengan masa juvenil singkat.

CTV seperti induknya. Identitas true-to-type regeneran terlihat dengan keberadaan pita DNA yang sama antara regeneran hasil kultur dengan pita DNA tanaman tetua.

Embrio somatik hasil perlakuan yang menghasilkan tanaman yang mempunyai potensi tingkat juvenile singkat, bebas penyakit dan true-to-type ini pada tahun kedua penelitian akan diperbanyak dengan menggunakan sistem kultur bioreaktor. Penggunaan bioreaktor dengan medium cair untuk perbanyakan tanaman sangat menguntungkan karena kemudahan *scaling-up*, kemampuan untuk mencegah gangguan fisiologi pucuk dan hiperhidrisitas daun dan biaya produksi rendah. Perbanyakan skala besar menggunakan bioreaktor juga bermanfaat karena produksi propagul sepanjang tahun, biaya murah dan waktu lebih singkat. Sehingga dengan didapatkannya metode perbanyakan masal bibit dengan sistem bioreaktor ini dan bibit diharapkan akan diperoleh bibit jeruk dengan masa juvenile singkat secara masal dan kontinyu. Sehingga pada tahun ketiga penelitian telah dapat disediakan bibit jeruk yang siap dipasarkan pada kelompok petani dan penangkar bibit serta menjalankan usaha penyediaan bibit dalam skala besar.

SUMMARY

Citrus fruit demand in Indonesia is increasing continuously while the production is declining from year to year. In order to provide for fulfillment of national consumption and of industrial raw materials, it needs developing an appropriate cultivation, one of those is the availability of quality seed in large quantities and uniform. In vitro propagation of citrus was carried out using somatic embryogenesis, however the technique ensures only the propagation of citrus rootstock, since these plants derived from somatic embryogenesis has a long juvenile period. Therefore, to overcome the problems mentioned above, it needs to be developed in vitro propagation technology of citrus stem with short juvenile period.

The research objectives for 3 years are to develop in vitro propagation technology of citrus stem with short juvenile period, to develop mass propagation technology of citrus seed by using bioreactor culture, to obtain superior seed without or with short juvenile period, and to develop superior seed supplier as well as citrus seed supplier industry.

Development of micropropagation technology of citrus scion with short juvenile period were conducted by modification of medium and culture condition. Evaluation of juvenility level was carried out by observation spine in plant 3 month after grafted to citrus rootstock. Identification of true-to-type genetic identity and virus free of regenerants were done by using SSR moleculer marker and by using ELISA. While large-scale propagation of citrus seed with short juvenile period, virus free and true-to-type will be conducted by bioreactor culture system. In order to retail citrus seed, some activities will be done, including identification of nurseries and farmer group of citrus, designing marketing communication to targeted nurseries and farmer, socialization and marketing collaboration to citrus nurseries and farmer group.

Expected output for 3 years are micropropagation technology of citrus with short juvenile period, virus free and true-to-type, technology of mass production of citrus seed by bioreactor culture system, population of citrus scion seed with short juvenile period, and stock industry of superior citrus seed, national and international scientific publication, intellectual property right related to propagation technology of citrus with short juvenile period.

In the first year of research were conducted: the development of methods to shorten the juvenile period in citrus resulted form somatic embryogenesis by modification medium and culture condition, maturation of treated somatic embryo and plant regeneration, grafting plantlet with Javanche Citroen (JC) root stock, evaluation juvenility level of regenerants, analysis of virus free by using ELISA, and analysis of true-to-type regenerants by using SSR molecular marker.

The research result showed that modification of culture medium and condition to shorten juvenility level of regenerant could affect somatic embryo growth. Addition of

sucrose up to 50 g/L in medium was able to raise somatic embryo fresh weight, but somatic embryo fresh weight start to reduce on adding of sucrose above 50 g/L. In contrast with sucrose, increasing of gibberelin in medium inhibit somatic embryo growth. The higher of gibberelin that added in medium, the lower somatic embryo fresh weight. Photoperiod also influence embryo somatic growth. Increasing of somatic embryo fresh weight as well as increasing photoperiod up to 20 hour/day. Contrary, the longer duration of somatic embryo incubation in cold temperature, the higher of reducing somatic embryo fresh weight.

Embryo maturation and germination simultaneously to obtain plantlet is a critical period of in vitro embryogenesis. Addition of medium with sucrose and extract malt was able to raise citrus somatic embryo maturation. Addition of medium with 50 gL^{-1} sucrose or 500 mgL^{-1} malt extract was the best medium for somatic embryo maturation. The type and sugar concentration that added in medium also influence somatic embryo maturation. Galactose was able to increase somatic embryo maturation better than maltose and sorbitol. By using combination of galactose and sorbitol or maltose and sorbitol were able to increase citrus somatic embryo maturation. Combination of galactose and sorbitol were able to increase somatic embryo maturation higher than maltose and sorbitol combination. Sorbitol 36.5 mM that combined with galactose and combination of 73 mM sorbitol with maltose produce somatic embryo maturation better than other concentration. Majority of somatic embryo growth in this medium is heart and torpedo stage, while cotyledonary stage was rare. However, the three kinds of embryo stages were able to form plantlet if it is cultured in germination media.

Plantlets regenerated from the somatic embryo have grafted with Javanche Citroen (JC) rootstock. Some of minigrafting plants showed short juvenile period, virus free and true-to-type. ELISA analysis from regeneration plant revealed that some plant negatively/not infected by Citrus Tristeza Virus (CTV) and some plant positively infected CTV like its parent. Identity of regeneration plant true-to-type can recognized by the presence of parent plant DNA bands.

Treated somatic embryo that produced plants has potency short juvenile period, virus free, and true-to-type will be propagated using mass bioreactor culture system in the second year. Plant propagation using bioreactor system should be an advantageous method in term of ease of scaling up, ability preventing physiological disturbance of shoot and hyperhydricity and low cost production. Large-scale micropropagation using bioreactor is also beneficial for seed production throughout the year, low cost and short time. Realizing mass propagation method of citrus seed by using bioreactor culture system will be obtained citrus seed, which have short juvenile period in large amount and continuously. Therefore in the third year of research, marketed citrus seed to farmer community can be provided and also seed supplier industry can be developed.

KESIMPULAN

Medium dengan penambahan sukrosa dan ekstrak malt mampu meningkatkan maturasi embrio somatic jeruk. Media dengan penambahan sukrosa 50 g/L atau ekstrak malt 500 mg/L merupakan medium terbaik untuk maturasi embrio somatik jeruk. Sebagian besar perkembangan embrio somatik hanya sampai pada tahapan torpedo, sedangkan untuk fase hati dan kotiledon jarang dijumpai. Namun demiklan embrio somatik fase torpedo tersebut mampu membentuk plantlet jika dikulturkan pada media perkecambahan.

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