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Multiplication of *Rubus* Germplasm *In Vitro*: A Screen of 256 Accessions

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Abstract

Rubus germplasm at the National Clonal Germplasm Repository, Corvallis, Oregon, was screened to determine tissue culture growth media suitable to the diverse collection being maintained. Explants were taken from mature pot-grown plants. Murashige and Skoog (MS) medium with 1 mg/l benzyladenine (BA) and 0.1 mg/l indole butyric acid (IBA) was used for initiation (explant establishment) of 256 different accessions of *Rubus*. Evaluation was based on a three fold (3X) increase in the number of plantlets following three weeks on the medium. Following two initial transfers, those which did not multiply 3X in three weeks were divided into two groups based on the vigor of the clone. Group 1 (low multiplication but growing well) were placed on MS with 1 mg/l BA, 0.1 mg/l IBA and 0.1 mg/l GA_3 while Group 2 (low multiplication and poor growth) were grown on Anderson's medium with 1 mg/l BA and 0.1 mg/l IBA. Accessions which did not respond with 3X growth after two transfers of three weeks each were placed on media with additional modifications. The majority (62%) will multiply 3X in three weeks on MS medium with 1 mg/l BA, 0.1 mg/l IBA and 0.1 mg/l GA_3 while another 18% respond on Anderson's medium at the same hormone levels. The remaining 20% require BA at 2 mg/l, 0.1/mg/l IBA and GA_3 0.1 mg/l to produce 3X growth in three weeks. This is the first report of the *in vitro* culture of many of these *Rubus* species and cultivars. Chemical names used: N(-phenylmethyl)-1H-purin-6-amine (BA); Indole-3-butyic acid (IBA); Gibberellic Acid A_3 (GA_3).

Introduction

In vitro propagation in individual cultivars of raspberry and blackberry and optimal basal media and hormone concentrations are well documented (1, 2, 3, 4, 7). Most studies have developed media formulations for one to five cultivars. The development of

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a medium to support widely varied germplasm collections would require a long period of time if each accession (species or cultivar) were individually optimized. The development of a medium for the *in vitro* propagation of cultivars and species is important in the maintenance of large germplasm collections. This information would also be useful to those working with virus indexing, cold storage or exchange of *in vitro* material. Ideally all accessions would be grown on one medium (6); however the great amount of genetic diversity found in a collection of hundreds of species and cultivars of a genus such as *Rubus* make this unlikely. This report provides information on the response of 256 *Rubus* species and cultivars to modifications of two widely used media which allow for growth and multiplication *in vitro*.

Materials and Methods

Nodal cuttings (3 cm) of 5 year old pot-grown plants in the screenhouse collection of the National Clonal Germplasm Repository, Corvallis, Oregon were surface sterilized by immersion for 15 min. in a 10% bleach solution (5.25% sodium hypochlorite) with 0.1 ml/l of Tween 20 followed by two sterile water rinses. Cuttings of all accessions were initiated in 16 mm tubes on Murashige and Skoog (MS) medium (5) with 0.1 mg/l indole-3-butyric acid (IBA), 1 mg/l Benzyladenine (BA) and 7 g/l agar (Difco-Bacto) with a pH of 5.7 before autoclaving. Growth room conditions were 16 hr days and 8 hr nights at 25C. Light intensity was 25 μEm^2s^{-1} at the level of the plants.

Cuttings were grown in the initiation medium for two transfers of three weeks each then evaluated for growth response (Fig. 1). Those which were multiplying at a rate of 3X or better per three week transfer period were cold stored at this point. Those which did not multiply at 3X were divided into two groups based on plant vigor. Group 1 (low multiplication but growing well) remained on the MS medium used for initiation and GA_3 at 0.1 mg/l was added. Group 2 (low multiplication and poor growth) were transferred to Anderson's raspberry medium (1). Two more growth cycles (3 weeks each) were run and cultures multiplying at 3X were cold stored. Group 1 clones which did not respond were transferred to MS medium with 2 mg/l BA, 0.1 mg/l IBA and 0.1 mg/l GA_3. This improved multiplication for some and they were stored. For group 2 clones which did not respond to Anderson's medium, GA_3 was added at 0.1 mg/l. Those that did not respond after two more three week transfers had an additional 1 mg/l BA added to the medium.

Cultures of 45 rapidly growing accessions were transferred to hormone-free MS medium for rooting. Not all accessions were tested for rooting ability due to time and space limitations.

Results

Nearly all accessions initiated could be multiplied using the medium modifications used in this study. The majority of accessions (69%) could be successfully cultured on one of the MS media formulations (Tables 1 A, B, C). Most of the accessions on this medium (Group 1) were blackberries (68%). The addition of GA_3 to the medium increased multiplication of some accessions (Table 1B). The accessions listed in Tables 1A and 2A were found to grow equally well with the addition of GA_3. The addition of 2 mg/l BA was needed to stimulate multiplication in others (Table 1C).

Anderson's medium with and without modifications (Group 2) improved multiplication for 34% of the accessions, of which 94% are raspberries (Tables 2 A, B, C). Some accessions remained alive but multiplied very slowly despite medium modifications (Table 2C).

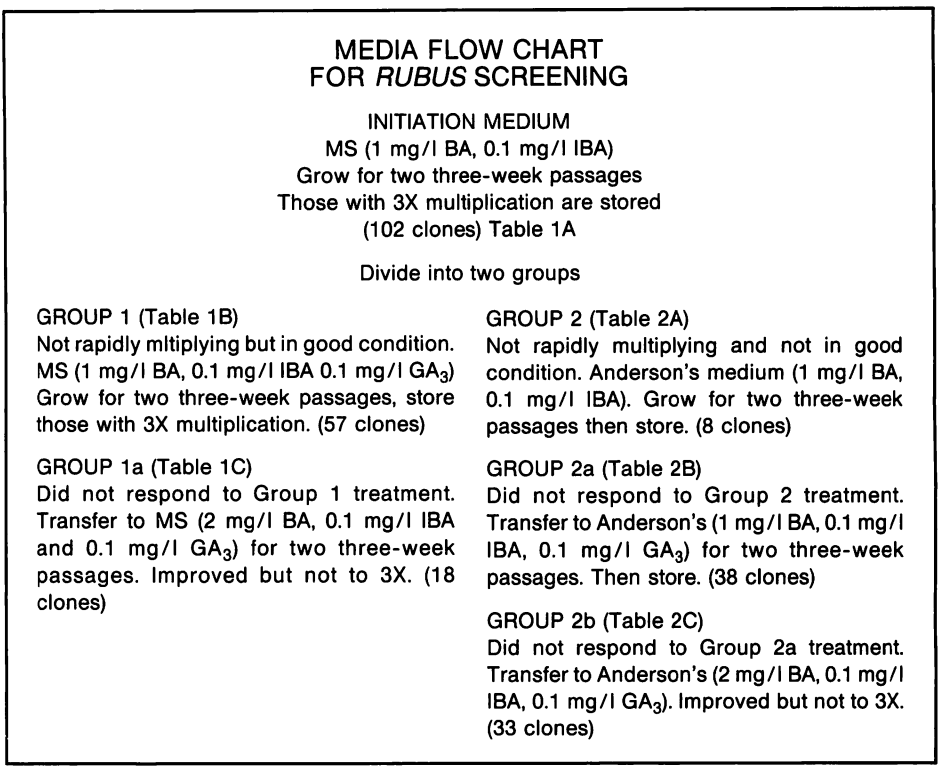

Figure 1.

Specific cultivars responded in a manner similar to previous reports. 'Black Satin' and 'Smoothstem' (Table 1A) had high multiplication rates on MS medium but did not require the addition of GA_3 as used by Broome and Zimmerman (2) and others (4). 'Bedford Giant' and 'Tayberry' cultured in Lindsmeier and Skoog medium by Harper (3) responded in a similar fashion respectively to 'Bedford Giant' (Table 1A) and 'Tayberry' seedling (Table 1B). The four cultivars of thornless blackberry grown in the Broome and Zimmerman study all grew well while in this study three other thornless blackberries, 'Thornless Logan,' 'Chester Thornless' and 'Thornfree' were recalcitrant.

Snir (7) successfully grew meristems of 'Heritage,' 'Malling Exploit' and 'September' (multiplication rates not given) on Boxus medium with MS micronutrients. With these three cultivars we found Anderson's medium with GA_3 superior to MS, with higher BA levels (2 mg/l) required for 'Heritage' and 'Malling Exploit' (Table 2C). Three cultivars also studied by Anderson (1) were included in this screen. Of those three, 'Willamette' (Table 1A) required less BA (1 mg/l) and no GA_3 compared to Anderson's study. 'Nootka' and 'Heritage' (Table 2C) grew best at the same hormone levels (2 mg/l BA, 0.1 mg/l GA) noted by Anderson (1).

Removal of all hormones from MS medium stimulated rooting in the 45 accessions tested (Table 3). All of the accessions listed in Table 3 rooted within the first 3 weeks following

Table 1A. *Rubus* accessions from the National Clonal Germplasm Repository (NCGR) with greater than 3X multiplication in three weeks on Murashige and Skoog medium with 1 mg/l BA and 0.1 mg/l IBA (102 clones).

Plant name	Crop type	NCGR Accession number
Anderson	Blackberry	393
Ashton Cross	Blackberry	317
Aurora	Blackberry	955
Austin Thornless	Blackberry	357
Bailey	Blackberry	62
Bedford Giant	Blackberry	312
Black Satin	Blackberry	151
Boysen	Blackberry	63
Brazos	Blackberry	64
Burbank Thornless	Blackberry	250
Carolina	Blackberry	956
Cascade	Blackberry	66
Chehalem	Blackberry	761
	Blackberry	203
Cherokee	Blackberry	67
Dirksen Thornless	Blackberry	71
Dyke	Blackberry	139
Ebano	Blackberry	833
Ebony King	Blackberry	73
Hillemeyer	Blackberry	252
	Blackberry	137
Kotata	Blackberry	359
Marion	Blackberry	385
NC110	Blackberry	362
ORUS 1063	Blackberry	346
ORUS 1280	Blackberry	366
ORUS 1465	Blackberry	350
ORUS 1467	Blackberry	345
ORUS 1600	Blackberry	369
ORUS 1620	Blackberry	348
ORUS 998	Blackberry	351
Raven	Blackberry	77
Smoothstem	Blackberry	80
Snyder	Blackberry	210
Waldo	Blackberry	983
Watlab	Blackberry	26
Whitford Thornless	Blackberry	722
Womack	Blackberry	205
R. alumnus Bailey	R. species	844
R. armeniacus Focke	R. species	45
R. caesius open pollinated hybrid	R. species	968
R. calcynoides Hayata	R. species	485

Table 1A. *(Continued).*

Plant name	Crop type	NCGR Accession number
R. discolor Weihe & Nees	R. species	818
R. errabundus Watson	R. species	40
R. flosculosus Focke	R. species	424
R. fruiticosus L.	R. species	248
R. fuscus Weihe & Nees	R. species	43
R. hirsutus Thunb.	R. species	959
	R. species	1
R. Hirtus Waldst. & Kit.	R. species	51
R. hispidus L.	R. species	794
R. idaeus v. strigosus (Michaux) Maxim.	R. species	183
R. illecebrosus Focke	R. species	838
R. lambertianus Ser.	R. species	181
R. lasiostylus Focke	R. species	430
R. lasiostylus v. hubeiensis	R. species	426
R. leucodermis Douglas ex Torrey & A. Gray	R. species	14
	R. species	655
R. microphyllus L. f.	R. species	158
R. odoratus L.	R. species	15
	R. species	11
R. parviflorus Nutt.	R. species	200
	R. species	199
R. parvifolius L.	R. species	776
	R. species	6
	R. species	29
	R. species	5
R. pungens Cambess.	R. species	966
R. rigidus Smith	R. species	144
R. rosaefolius Smith	R. species	188
	R. species	486
R. shankii Standley & L. O. Williams	R. species	41
R. shankii Standley & L. O. Williams	R. species	145
R. sp.	R. species	967
	R. species	635
	R. species	419
	R. species	140
	R. species	49
	R. species	22
R. spectabilis Pursh	R. species	255
	R. species	202
	R. species	961
	R. species	256
R. Spectabilis v. menziesii Pursh	R. species	4

Table 1A. ***(Continued).***

Plant name	Crop type	NCGR Accession number
R. sumatranus Miq.	R. species	7
R. thyrsoideus Wimmer	R. species	33
	R. species	965
R. tomentosus Borkh.	R. species	36
R. trivialis Michaux	R. species	421
R. ulmifolius f. bellidiflorus Voss	R. species	34
R. ulmifolius open pollinated hybrid	R. species	23
R. ursinus Chamb. & Schldl.	R. species	804
	R. species	615
Baumforth (Standard A)	Raspberry	155
Boyne	Raspberry	118
Malling Promise	Raspberry	444
NC 84-10-8 (R. occidentalis L.)	Raspberry	730
NC 86-14-02	Raspberry	988
ORUS 1308	Raspberry	970
ORUS 963	Raspberry	361
Veten	Raspberry	454
Willamette	Raspberry	100

Table 1B. *Rubus* accessions from the National Clonal Germplasm Repository (NCGR) with greater than 3X multiplication in three weeks on Murashige and Skoog medium with 1 mg/l BA, 0.1 mg/l IBA and 0.1 mg/l GA_3 (57 clones).

Plant name	Crop type	NCGR Accession number
Aurora	Blackberry	134
Cheyenne	Blackberry	68
Eldorado	Blackberry	103
Hull Thornless	Blackberry	389
Kotata	Blackberry	992
Merton Thornless	Blackberry	254
ORUS 1127	Blackberry	344
ORUS 1278	Blackberry	458
ORUS 742	Blackberry	352
ORUS 965	Blackberry	349
Oklawaha	Blackberry	720
Olallie	Blackberry	76
Philadelphia	Blackberry	207
Santiam	Blackberry	79
Silvan	Blackberry	633
Thornless Oregon Evergreen	Blackberry	991
	Blackberry	82

Table 1B. ***(Continued).***

Plant name	Crop type	NCGR Accession number
Tayberry seedling (not Cultivar)	Hybrid	227
R. caesius L.	R. species	19
R. caesius open pollinated hybrid	R. species	150
R. canadensis L.	R. species	25
	R. species	196
R. cissoides Cunn.	R. species	814
R. distractiformis Newton	R. species	219
R. idaeus L.	R. species	238
R. lasiococcus A. Gray	R. species	612
R. lasiostylus Focke	R. species	427
R. leucodermis Douglas ex Torrey & A. Gray	R. species	647
	R. species	653
	R. species	599
R. nessensis Hall	R. species	832
R. parviflorus Nutt.	R. species	800
	R. species	52
R. plicatus Weihe & Nees	R. species	44
R. sp.	R. species	132
R. thyrsoideus Wimmer	R. species	48
R. thyrsoideus Wimmer	R. species	37
R. trivialis Michaux	R. species	982
	R. species	724
R. ulmifolius f. bellidiflorus Voss	R. species	813
R. ursinus Cham. & Schldl.	R. species	611
Bendender	Raspberry	816
Colossus	Raspberry	996
Gradinia	Raspberry	1000
Krupna Dvorda	Raspberry	1001
M-52-71 (Serbia)	Raspberry	146
Malling Enterprise	Raspberry	1003
NC 84-10-4 (R. occidentalis L.)	Raspberry	726
NC 84-10-5 (R. occidentalis L.)	Raspberry	727
NC 84-10-7 (R. occidentalis L)	Raspberry	729
ORUS 1028	Raspberry	277
ORUS 1314	Raspberry	289
ORUS 1341	Raspberry	292
Pocahontas	Raspberry	1004
Puyallup	Raspberry	489
Trent	Raspberry	380
Zzopska Alena	Raspberry	456

Table 1C. *Rubus* accessions from the National Clonal Germplasm Repository (NCGR) with multiplication rates less than 3X in three weeks on MS media with 2 mg/l BA, 0.1 mg/l IBA and 0.1 mg/l GA_3 (18 clones).

Plant name	Crop type	NCGR Accession number
Chester Thornless	Blackberry	839
Comanche	Blackberry	69
Flordagrand	Blackberry	721
ORUS 1067	Blackberry	368
ORUS 922	Blackberry	360
ORUS 992	Blackberry	347
Thornfree	Blackberry	105
Thornless Logan	Blackberry	81
Young	Blackberry	131
R. hirsutus Thunb.	R. species	8
R. hirtus Waldst. & Kit.	R. species	35
R. lambertianus var. glabra	R. species	423
R. parviflorus Nutt.	R. species	61
R. ursinus Cham. & Schldl.	R. species	197
Chief	Raspberry	995
Lowden	Raspberry	433
Malling Orion	Raspberry	1002
ORUS 1029	Raspberry	288

Table 2A. *Rubus* accessions from the National Clonal Germplasm Repository (NCGR) with greater than 3X multiplication in three weeks on Anderson's medium with 1 mg/l BA and 0.1 mg/l IBA (8 clones).

Plant name	Crop type	NCGR Accession number
R. lasiococcus A. Gray	R. species	261
R. lasiostylus Focke	R. species	327
R. parviflorus Nutt.	R. species	609
R. scanicus Chapple & Watson	R. species	39
Blackhawk	Raspberry	84
Bristol	Raspberry	85
Matsqui	Raspberry	215
Southland	Raspberry	1005

Table 2B. *Rubus* accessions from the National Clonal Germplasm Repository (NCGR) with greater than 3X multiplication in three weeks on Anderson's medium with 1 mg/l BA, 0.1 mg/l IBA and 0.1 mg/l GA_3 (38 clones).

Plant name	Crop type	NCGR Accession number
Bodega Bay (R. ursinus Cham. & Schldl.)	Blackberry	367
Chehalem	Blackberry	133
ORUS 1362	Blackberry	459
R. calcynoides Hayata	R. species	179
R. crataegifolius Bunge	R. species	268
R. idaeus L.	R. species	591
R. idaeus L.	R. species	239
R. idaeus v. peramoenus (E. Greene) Fern	R. species	607
R. idaeus v. strigosus (Michaux) Maxim.	R. species	17
R. lasiococcus A. Gray	R. species	258
R. muelleri Lef.	R. species	762
R. parviflorus Nutt.	R. species	13
R. phoenicolasius Maxim.	R. species	163
R. trivialis Michaux	R. species	260
Amity	Raspberry	632
Canby	Raspberry	384
Carnival	Raspberry	492
Chilliwack	Raspberry	985
Glen Esk	Raspberry	321
Heija	Raspberry	263
Jingu Juegal (R. crataegifolius Bunge)	Raspberry	267
Jokgal (R. crataegifolius Bunge)	Raspberry	449
Madawaska	Raspberry	126
Mandarin	Raspberry	743
Marcy	Raspberry	377
ORUS 1153	Raspberry	278
ORUS 1402	Raspberry	305
ORUS 1510	Raspberry	302
ORUS 1699	Raspberry	460
ORUS 892	Raspberry	273
Prestige	Raspberry	387
Rubin Bulgarski	Raspberry	451
September	Raspberry	127
Shuttleworth	Raspberry	106
Summit	Raspberry	984
Tahoma	Raspberry	453
WYO US 68-21	Raspberry	1014
Zefa [Zeva Remontante]	Raspberry	455

Table 2C. *Rubus* accessions from the National Clonal Germplasm Repository (NCGR) with multiplication rates less than 3X in three weeks on Anderson's medium with 2 mg/l BA, 0.1 mg/l IBA and 0.1 mg/l GA_3 (33 clones).

Plant name	Crop type	NCGR Accession number
Brison	Blackberry	65
Thornless Logan	Blackberry	774
R. lasiostylus Focke	R. species	425
R. palmatus Thunb.	R. species	2
R. parvifolius L.	R. species	184
	R. species	147
R. sp.	R. species	178
Comox	Raspberry	986
Defiance	Raspberry	447
East Malling 3655/47	Raspberry	229
Fairview	Raspberry	488
Festival	Raspberry	491
Goldenwest	Raspberry	440
Haida	Raspberry	95
Heritage	Raspberry	990
Jewel (Black Raspberry)	Raspberry	88
Killarney	Raspberry	493
M-51-71 (Serbia)	Raspberry	141
Malling Delight	Raspberry	319
Malling Exploit	Raspberry	316
Malling Jewel	Raspberry	773
Malling Landmark	Raspberry	96
Malling Leo	Raspberry	318
Munger	Raspberry	490
NC 84-10-3 (R. occidentalis L.)	Raspberry	725
NC 85-8-2 (R. idaeus v. strigosus (Michaux)	Raspberry	738
Nootka	Raspberry	122
ORUS 769	Raspberry	373
Pathfinder	Raspberry	392
Sentry	Raspberry	209
St. Walfried	Raspberry	372
Sumner	Raspberry	382
Viking	Raspberry	115

Table 3. *Rubus* accessions from the National Clonal Germplasm Repository (NCGR) which rooted within three weeks of transfer to Murashige and Skoog medium without hormones.

Plant name	Crop type	NCGR Accession number
Aurora	Blackberry	955
Austin Thornless	Blackberry	357
Brazos	Blackberry	64
Burbank Thornless	Blackberry	250
Chehalem	Blackberry	133
NC110	Blackberry	362
ORUS 1127	Blackberry	344
ORUS 1620	Blackberry	348
ORUS 998	Blackberry	351
Waldo	Blackberry	983
R. armeniacus Focke	R. species	45
R. fruiticosus L.	R. species	248
R. hirsutus Thunb.	R. species	1
R. hirsutus Thunb.	R. species	959
R. hirtus Waldst. & Kit.	R. species	51
R. hispidus L.	R. species	794
R. lasiococcus A. Gray	R. species	261
R. lasiostylus Focke	R. species	327
R. leucodermis Douglas ex Torrey & A. Gray	R. species	653
R. leucodermis Douglas ex Torrey & A. Gray	R. species	14
R. odoratus L.	R. species	11
R. odoratus L.	R. species	15
R. palmatus Thunb.	R. species	2
R. parviflorus Nutt.	R. species	609
R. parviflorus Nutt.	R. species	199
R. parviflorus Nutt.	R. species	200
R. parviflorus Nutt.	R. species	800
R. parvifolius L.	R. species	6
R. rigidus Smith	R. species	144
R. rosaefolius Smith	R. species	188
R. scanicus Chapple & Watson	R. species	39
R. shankii Standley & L. O. Williams	R. species	145
R. shankii Standley & L. O. Williams	R. species	41
R. sp.	R. species	967
R. sp.	R. species	49
R. spectabilis Pursh	R. species	961
R. spectabilis Pursh	R. species	202
R. spectabilis Pursh	R. species	255

Table 3. ***(Continued).***

Plant name	Crop type	NCGR Accession number
R. spectabilis v. menziesii Pursh	R. species	4
R. sumatranus Miq.	R. species	7
R. ursinus Chamb. & Schldl.	R. species	804
Heija	Raspberry	263
Matsqui	Raspberry	215
ORUS 1308	Raspberry	970
Zzopska Alena	Raspberry	456

transfer onto MS medium without hormones. Other slower growing *Rubus* cultivars may require the addition of IBA to stimulate rooting (4).

Discussion

This screen has demonstrated that selecting a medium for multiplying large numbers of species and cultivars in the genus *Rubus* can be accomplished without optimizing media for each accession. Among the 256 accessions of *Rubus* used in this study are some that are very diverse genetically and have originated from various parts of the world. Most have not previously been grown *in vitro*. Differences between the results of this study and earlier work may be due to the age of the source plants or the season of initiation. Six week, rather than longer, culture periods were used in this study since most modifications involved adding one new component to standard media rather than deleting components. Relatively fast changes were necessary to keep struggling accessions alive until the proper medium was found.

To optimize medium requirements for this many accessions would require years of study. By using a screen starting with one or two basal media and modifying the hormone concentration with large groups of accessions, the time required from initiation to final storage for most of the accessions of this genus should be three to five months. Those that remain recalcitrant should be reinitiated and optimized individually.

We have successfully used this procedure to initiate and grow these 256 *Rubus* accessions. This information on the basal medium required for *Rubus* species and cultivars provides a starting point for those wishing to optimize the medium for a particular *Rubus* cultivar or species.

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