Variation in Root Development Response to flooding among 92 Soybean Lines during Early Growth Stages

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Variation in Root Development Response to Flooding among 92 Soybean Lines during Early Growth Stages

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Abstract: Maintaining root function is crucial for favorable plant growth under flooding. The genetic variation in the response of root development to flooding is unclear, because measurement of root growth is time consuming, especially with numerous lines. To overcome the methodological problems and to reveal the effect of flooding on root development and its genetic variation, we developed a new capillary watering system without soil medium and raised cotyledon-stage seedlings of 92 soybean lines with and without flooding. After 7 days of flooding, dry weights (DW) and root characteristics were determined and the results were compared with those in non-flooded plants. The root DW decreased linearly with decreasing total root length and root surface area, and the degree of damage varied greatly among lines. Short-term flooding inhibited root elongation and branching, but not in flood-tolerant lines.

Key words: Early growth stage, Flood-tolerance, Genetic variation, Root development, Soybean (*Glycine max*).

Both natural and anthropogenic flooding cause yield losses in agriculture (Rosenzweig et al., 2002). Like most crops, soybean (*Glycine max* (L.) Merr.), which is the most widely grown legume in the world, is susceptible to flooding, which reduces plant biomass and yield (Sugimoto et al., 1988; Scott et al., 1989; Linkemer et al., 1998; Bacanamwo and Purcell, 1999a; Henshaw et al., 2007a, b; Rhine et al., 2010). In Eastern Asia, soybean is often grown in upland fields converted from paddy fields (Lee et al., 2003; MAFF, 2004), and is generally sown in the spring to early summer monsoon season, placing the seedlings at risk of flood damage. Even short-term flooding can inhibit or kill seedlings, leading to serious yield losses.

Because roots acquire nutrients and water and synthesize organic acids, amino acids, and plant hormones (Yang et al., 2004), root development is closely related to aboveground development (Yang et al., 2012). Flooding damages both root and shoot growth (Sallam and Scott, 1987; Araki et al., 2012), affecting root growth first (Sauter, 2013); for example, in soybean, flooding reduced root dry weight (DW) before it reduced shoot DW (Shimamura et al., 2003a). Therefore, maintaining root development is crucial for favorable plant growth under flooding.

Internal oxygen transport from the air to the roots is important for root survival and function (Armstrong, 1979). Flooded plants often suffer hypoxia or anoxia stress, because oxygen moves much more slowly in water ($\times 10^{-4}$) than in the air (Armstrong, 1979; Armstrong and Drew, 2002), and dissolved oxygen is quickly depleted: the oxygen concentration in a nutrient solution decreased by about 80% within 24 hr after transfer of wheat seedlings into the solution (Wiengweera et al., 1997). Under prolonged flooding, normal root development is replaced by the formation of adventitious roots in field crops such as wheat (Mano and Omori, 2007) and tomato (McNamara and Mitchell, 1990). Flood-tolerant species often form adventitious roots from submerged stems (Colmer and Voesenek, 2009). Removal of adventitious roots from the wetland plants *Cotula coronopifolia* and *Meionectes brownii* under flooding reduced whole-plant growth, indicating that the adventitious roots are of some benefit to plant growth during flooding (Rich et al., 2012). Soybean plants can develop adventitious roots *de novo* during flooding, but it takes time for the roots to form and function effectively: roots grew only 1 cm after 4 to 5 d (Thomas et al., 2005). During the early growth stages, flood-tolerant rice

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Abbreviations: ANOVA, analysis of variance; DW, dry weight.

Table 1. Average dry weight (mg ± SD) in 92 soybean lines at the cotyledon stage.

Lines[Y]	Root DW		Shoot DW		Whole-plant DW	
	Flooding	Control	Flooding	Control	Flooding	Control
Kitamusume	65.0 ± 12.24	120.7 ± 21.30	132.0 ± 39.34	128.1 ± 27.04	197.0 ± 51.28	248.9 ± 48.33
Toyokomachi	77.1 ± 17.94	141.9 ± 35.03	185.7 ± 86.99	181.2 ± 51.47	262.8 ± 103.16	323.0 ± 86.50
SH20	64.9 ± 4.77	118.4 ± 0.60	133.4 ± 17.33	151.7 ± 11.43	200.8 ± 24.63	266.8 ± 17.41
Misuzudaizu	87.5 ± 30.39	156.8 ± 59.59	147.0 ± 61.20	154.3 ± 36.90	234.5 ± 91.33	311.0 ± 95.18
Koitozairai	82.9 ± 3.07	146.3 ± 3.80	117.5 ± 14.75	115.3 ± 1.51	200.4 ± 17.55	261.6 ± 4.72
Tachinagaha	71.7 ± 18.53	125.8 ± 32.20	89.8 ± 37.27	101.2 ± 18.11	161.5 ± 55.80	227.0 ± 49.63
COL/Aomori/1983-21	87.4 ± 3.91	149.4 ± 13.29	146.6 ± 28.75	127.8 ± 15.07	234.0 ± 31.87	277.2 ± 27.32
Toiku 241	58.8 ± 8.77	99.4 ± 10.19	114.5 ± 26.87	115.7 ± 19.47	173.3 ± 35.64	215.1 ± 29.64
Fukuyutaka	86.3 ± 15.35	143.1 ± 20.73	118.1 ± 27.84	113.4 ± 1.49	204.3 ± 42.11	256.5 ± 20.26
Nasushirome	68.2 ± 8.54	109.3 ± 10.46	121.5 ± 13.23	124.8 ± 17.69	189.8 ± 20.28	234.1 ± 27.53
Ipponsango	72.2 ± 9.89	115.5 ± 1.39	102.4 ± 28.60	102.2 ± 6.25	174.6 ± 37.03	217.7 ± 5.14
Tozan 206	75.2 ± 16.76	120.1 ± 17.67	122.9 ± 21.08	110.3 ± 9.86	198.1 ± 37.82	230.4 ± 27.52
Shiroge 9	80.5 ± 16.60	126.6 ± 15.80	129.1 ± 29.06	123.7 ± 14.55	209.6 ± 45.48	250.2 ± 30.17
Toyohomare	69.1 ± 16.36	108.5 ± 46.48	122.8 ± 53.42	123.1 ± 43.66	191.9 ± 69.30	231.6 ± 89.86
Tanishidaizu	60.6 ± 24.21	94.2 ± 48.56	96.3 ± 57.34	91.4 ± 48.18	156.9 ± 81.08	185.6 ± 96.55
Kiyomidori	79.2 ± 9.02	123.0 ± 22.46	161.5 ± 30.23	131.0 ± 19.84	240.6 ± 39.06	254.0 ± 42.27
Hyuga	77.3 ± 19.07	118.8 ± 19.88	116.7 ± 27.31	111.0 ± 13.95	194.0 ± 46.12	229.8 ± 32.71
To-8E	92.1 ± 15.15	140.8 ± 30.67	146.6 ± 29.90	161.9 ± 39.89	238.7 ± 44.19	302.7 ± 70.50
Kosuzu	53.3 ± 2.37	81.5 ± 14.72	94.8 ± 2.99	93.3 ± 20.02	148.1 ± 5.07	174.8 ± 34.73
Nattoshoryu	48.1 ± 10.44	73.5 ± 18.97	87.4 ± 21.68	80.0 ± 16.52	135.5 ± 32.09	153.5 ± 35.31
Enrei	80.9 ± 15.78	123.6 ± 17.30	129.9 ± 15.55	128.9 ± 13.26	210.8 ± 30.97	252.4 ± 28.40
Hiden	77.6 ± 11.09	118.3 ± 25.99	119.9 ± 29.23	104.4 ± 33.14	197.5 ± 40.16	222.7 ± 58.52
Tamahomare	75.5 ± 6.40	114.8 ± 15.88	114.5 ± 11.26	122.3 ± 8.13	190.0 ± 17.65	237.1 ± 23.97
Toyomusume	69.5 ± 6.25	105.4 ± 19.79	122.7 ± 22.65	114.3 ± 10.58	192.2 ± 26.91	219.7 ± 30.07
Karikei 476	85.2 ± 11.30	128.4 ± 32.63	114.6 ± 34.51	117.4 ± 32.68	199.7 ± 44.38	245.8 ± 65.30
Tohoku 129	81.9 ± 4.70	122.8 ± 28.34	141.3 ± 12.11	125.3 ± 22.72	223.2 ± 8.51	248.1 ± 50.97
Norin No. 2	77.0 ± 10.33	114.9 ± 14.47	109.1 ± 8.89	107.8 ± 24.02	186.1 ± 1.61	222.7 ± 37.29
Ryuhou	86.4 ± 5.92	127.9 ± 11.51	126.0 ± 13.54	122.1 ± 17.20	212.4 ± 18.92	250.0 ± 28.60
Jizuka	43.3 ± 4.04	63.8 ± 11.21	69.8 ± 14.45	69.5 ± 13.60	113.0 ± 18.45	133.3 ± 24.62
Sachiyutaka	87.5 ± 13.26	124.4 ± 21.25	127.4 ± 49.48	112.2 ± 28.94	214.9 ± 62.46	236.6 ± 50.17
Tsurukogane	90.7 ± 13.43	128.6 ± 17.57	118.5 ± 24.20	126.3 ± 30.16	209.2 ± 36.63	254.9 ± 47.41
Toyosuzu	82.3 ± 14.89	116.3 ± 25.02	137.2 ± 25.55	124.0 ± 16.66	219.5 ± 39.88	240.3 ± 41.38
Hourei	73.1 ± 17.80	103.1 ± 28.98	98.4 ± 29.52	94.2 ± 20.21	171.5 ± 47.29	197.4 ± 49.18
Karikei 599	83.6 ± 8.63	117.5 ± 18.02	147.1 ± 36.37	119.5 ± 10.74	230.7 ± 44.99	237.1 ± 26.27
Akita	90.3 ± 3.74	126.8 ± 6.14	127.1 ± 10.81	109.6 ± 14.99	217.4 ± 13.45	236.4 ± 20.56
Nezumisaya	64.1 ± 13.19	89.9 ± 20.94	103.0 ± 32.91	99.6 ± 25.20	167.1 ± 45.98	189.4 ± 46.11
SUWEON 95	92.8 ± 10.28	129.9 ± 7.59	149.6 ± 29.46	144.5 ± 26.05	242.4 ± 39.53	274.3 ± 33.47
Nakasennari	88.0 ± 5.79	122.8 ± 20.35	117.5 ± 18.35	107.3 ± 19.68	205.5 ± 12.56	230.1 ± 37.55
Hayahikari	68.6 ± 9.22	95.4 ± 8.22	110.9 ± 27.28	91.1 ± 13.90	179.5 ± 34.96	186.5 ± 22.10
Tachiyutaka	66.7 ± 3.25	92.6 ± 9.00	83.6 ± 3.50	86.3 ± 7.88	150.3 ± 6.38	178.9 ± 16.01
Gedenshirazu	80.8 ± 25.18	111.9 ± 24.87	110.0 ± 29.79	117.7 ± 30.56	190.8 ± 54.39	229.6 ± 51.37
A100	74.3 ± 7.15	102.7 ± 27.29	83.1 ± 12.13	85.3 ± 22.67	157.4 ± 18.75	188.0 ± 49.64
Tokachinagaha	58.3 ± 7.15	80.5 ± 6.98	74.7 ± 17.97	64.6 ± 10.99	133.0 ± 24.93	145.1 ± 17.73
Waseshiroge	77.9 ± 9.72	107.0 ± 6.89	130.8 ± 19.93	109.4 ± 15.33	208.7 ± 29.08	216.4 ± 20.02
Chasengoku 81	68.1 ± 4.60	93.3 ± 17.25	78.7 ± 9.31	77.0 ± 26.67	146.8 ± 13.89	170.3 ± 43.91
Tokei 970	83.9 ± 4.98	114.9 ± 8.54	111.0 ± 11.27	99.0 ± 10.16	194.9 ± 16.23	213.8 ± 16.09
BIL17	63.2 ± 2.34	86.5 ± 0.40	71.3 ± 6.25	63.7 ± 4.04	133.7 ± 9.00	149.5 ± 5.12
KLS733-1	60.0 ± 19.66	81.9 ± 29.40	90.6 ± 35.12	82.9 ± 25.77	150.6 ± 54.77	164.8 ± 55.16
Yukihomare	65.8 ± 13.49	89.7 ± 26.00	87.5 ± 19.57	78.3 ± 23.83	153.3 ± 33.05	167.9 ± 49.80
Bay	78.0 ± 8.21	105.9 ± 12.18	97.9 ± 17.40	102.4 ± 24.44	175.9 ± 24.89	208.3 ± 36.57
Otofuke-ohsode	71.1 ± 4.04	96.1 ± 27.24	131.9 ± 7.15	104.4 ± 33.54	203.0 ± 10.34	200.5 ± 60.65
PRIZE	73.0 ± 6.89	98.1 ± 15.86	102.8 ± 25.65	93.3 ± 11.65	175.8 ± 32.24	191.3 ± 26.34
Norin No. 4	74.8 ± 5.20	99.9 ± 8.95	113.8 ± 3.20	79.3 ± 7.40	188.6 ± 8.13	179.2 ± 16.09
Kingen No. 1	61.5 ± 13.44	82.1 ± 19.10	102.1 ± 35.38	94.1 ± 27.45	163.6 ± 48.82	176.2 ± 45.90
Koganedaizu	56.7 ± 2.75	75.6 ± 19.37	83.1 ± 5.22	81.3 ± 14.00	139.9 ± 5.63	156.9 ± 29.63
Soudendaizu	52.9 ± 9.58	69.8 ± 16.51	83.9 ± 12.08	84.8 ± 6.29	136.8 ± 21.65	154.6 ± 22.54
Tokei 758	71.0 ± 2.03	93.0 ± 7.33	71.0 ± 3.84	71.7 ± 3.28	142.1 ± 4.91	164.6 ± 8.22
Williams 82	83.8 ± 6.21	109.5 ± 15.03	103.1 ± 11.57	101.9 ± 14.92	186.9 ± 17.54	211.4 ± 26.94
Aso-aogari	52.8 ± 2.13	68.7 ± 3.27	63.3 ± 1.95	59.7 ± 7.32	116.0 ± 3.79	128.4 ± 10.27
Ibarakimame 7	84.8 ± 17.81	110.4 ± 6.71	150.2 ± 47.42	111.2 ± 15.68	235.1 ± 65.22	221.6 ± 21.91
Fukuibuki	83.8 ± 3.23	108.8 ± 10.90	119.3 ± 11.01	119.9 ± 7.24	203.0 ± 14.04	228.7 ± 18.14
BRS154	100.3 ± 12.68	129.9 ± 13.28	114.7 ± 16.86	106.7 ± 16.25	215.0 ± 29.05	236.7 ± 29.52
Higomusume	50.2 ± 11.92	64.9 ± 18.00	75.5 ± 18.49	68.1 ± 23.03	125.7 ± 29.79	133.0 ± 41.02
COL/ Akita/1994/Kikuchi-1	50.9 ± 10.35	65.6 ± 12.92	79.0 ± 31.78	65.6 ± 11.89	129.9 ± 42.11	131.3 ± 24.77
Mizumoto park No. 3[Z]	12.0 ± 1.84	15.4 ± 1.60	17.0 ± 1.78	19.4 ± 3.91	29.0 ± 3.58	34.8 ± 5.22
Hougyoku	76.7 ± 3.24	98.5 ± 10.99	100.6 ± 1.41	82.2 ± 10.64	177.3 ± 4.58	180.7 ± 19.45
Ohsuzu	90.3 ± 11.99	115.9 ± 7.18	133.5 ± 8.49	112.8 ± 6.23	223.9 ± 20.48	228.7 ± 6.70
Kyushu 126	80.9 ± 14.56	102.9 ± 13.11	127.1 ± 45.78	121.6 ± 18.25	208.0 ± 60.10	224.5 ± 31.17
Tama-urara	89.1 ± 12.43	113.0 ± 14.69	152.9 ± 38.95	132.2 ± 9.93	242.0 ± 50.51	245.1 ± 24.42
Mizumoto park No. 2[Z]	8.5 ± 4.49	10.7 ± 5.59	11.0 ± 5.61	10.8 ± 5.23	19.5 ± 10.09	21.5 ± 10.74
Suzuyutaka	71.1 ± 11.41	88.9 ± 8.56	97.6 ± 5.52	94.6 ± 11.68	168.7 ± 16.43	183.4 ± 19.92
PI103091	55.2 ± 5.47	68.8 ± 16.98	79.9 ± 11.64	77.6 ± 23.36	135.0 ± 16.67	146.4 ± 39.34
Himeshiarzu	47.2 ± 2.00	57.3 ± 2.13	65.0 ± 9.64	57.0 ± 9.47	112.2 ± 10.94	114.3 ± 11.55
India (IC24527)	45.0 ± 8.80	54.4 ± 10.14	54.9 ± 20.25	42.4 ± 7.10	99.9 ± 29.05	96.8 ± 17.11
Adams	63.9 ± 15.40	77.2 ± 13.77	91.8 ± 31.38	79.7 ± 12.74	155.8 ± 46.73	156.9 ± 26.47
Koganejiro	73.9 ± 9.49	88.3 ± 8.42	89.3 ± 2.61	66.9 ± 5.60	163.3 ± 11.76	155.2 ± 5.39
Jack	67.4 ± 5.75	80.2 ± 5.18	89.0 ± 13.52	72.2 ± 7.33	156.4 ± 19.11	152.4 ± 12.42
Peking	47.0 ± 1.54	55.2 ± 7.51	65.4 ± 18.26	57.2 ± 11.52	112.4 ± 19.06	112.4 ± 19.01
COL/Korean/1995/Kikuchi-3 (white flower)	49.2 ± 12.45	57.4 ± 13.10	67.0 ± 16.55	57.7 ± 25.08	116.3 ± 28.55	115.1 ± 38.17
Tanyou	62.3 ± 5.43	72.6 ± 12.38	81.1 ± 7.39	70.5 ± 6.01	143.4 ± 12.75	143.1 ± 18.00
Mizumoto park No. 4[Z]	15.1 ± 4.12	17.4 ± 3.66	23.2 ± 7.14	22.5 ± 6.15	38.3 ± 11.25	39.9 ± 9.72
Kinusayaka	83.4 ± 3.74	95.9 ± 10.78	110.8 ± 12.21	100.0 ± 22.72	194.2 ± 15.95	195.9 ± 26.56
COL/PAK/1989/IBPGR/2326 (1)	39.0 ± 7.79	44.5 ± 7.46	41.8 ± 9.00	41.2 ± 10.88	80.8 ± 16.66	85.7 ± 18.23
Moshidou Gong 503	32.2 ± 5.14	36.6 ± 2.99	36.1 ± 7.45	35.3 ± 6.84	68.4 ± 12.18	71.9 ± 9.80
Harosoy	63.2 ± 2.42	70.8 ± 2.62	68.3 ± 5.08	58.3 ± 6.94	131.5 ± 6.47	129.1 ± 8.97
Wilis	46.4 ± 17.88	51.5 ± 12.07	68.1 ± 38.37	50.9 ± 13.01	114.5 ± 56.25	102.5 ± 24.54
Syokkou 9901	63.5 ± 16.84	69.1 ± 2.27	110.6 ± 38.23	91.0 ± 3.18	174.1 ± 55.04	160.2 ± 4.23
Tohoku 152	78.8 ± 13.66	84.1 ± 19.94	111.9 ± 40.61	98.2 ± 27.89	190.7 ± 53.59	182.3 ± 47.70
Mizumoto park No. 1[Z]	10.2 ± 0.90	10.8 ± 1.36	13.5 ± 0.31	13.4 ± 2.44	23.7 ± 1.21	24.2 ± 3.77
TH112-1	50.9 ± 15.95	53.5 ± 4.27	72.7 ± 31.06	55.3 ± 4.85	123.6 ± 46.89	108.7 ± 8.94
Iyodaizu	62.4 ± 8.63	63.3 ± 5.08	83.7 ± 3.70	74.0 ± 8.44	146.1 ± 11.68	137.3 ± 12.16
G406[Z]	8.2 ± 1.56	8.2 ± 1.43	10.2 ± 2.46	9.2 ± 0.62	18.4 ± 4.02	17.5 ± 1.93

[Z] *G. soja*

[Y] ranked according to the rate of inhibition of root DW genotypes were able to maintain their root growth under flooding (Ismail et al., 2009). As the disruption of early root development can restrict plant growth, flood-tolerant soybeans need to maintain root development. Therefore, to identify flood-tolerant soybeans, it is necessary to measure root growth. However, most plant scientists are reluctant to work in root morphology because root measurement is time consuming and it is difficult to measure root traits in a large number of lines (Waines and Ehdaie, 2007). Previous studies of soybean roots have been limited to just primary root length or biomass (Bacanamwo and Purcell, 1999a; Shimamura et al., 2003a; Henshaw et al., 2007a; Hashiguchi et al., 2009), and genetic variation in root development of flooded seedlings remains unclear.

To examine the effect of flooding on root development and its genetic variation, we raised seedlings of 92 soybean lines using a new capillary watering system instead of using soil, and measured shoot and root dry weight after 7 d with or without flooding. To identify important root traits responsible for flood tolerance, we correlated the rate of inhibition of root dry weight with the rates of inhibition of total root length, root surface area, and average root diameter.

Materials and Methods

1. Plant materials

We tested 92 soybean lines: 87 *Glycine max* and 5 *Glycine soja* Sieb. et Zucc. (Table 1). All belong to the *Glycine* subgenus *Soja*. *Glycine soja* is a progenitor of *Glycine max* (Hymowitz and Newell, 1980), and is often found in marshy areas such as riverbanks and lakesides, in disturbed sites, and on mountain slopes (Jin et al., 2006). Seeds of each line except Mizumoto Park Nos. 1 – 4 were collected at the National Institute of Crop Science, Tsukuba, Japan (36º 1´N, 140º 5´E), in 2008; and seeds of Mizumoto Park Nos. 1 – 4 were collected from a natural population in Mizumoto Park, Tokyo, Japan (35º 79´N, 139º 87´E), in 2009. All seeds were stored at 4ºC until our experiments.

2. Capillary watering culture system

For each line, 20 seeds coated with a fungicide (benomyl, 0.5% of dry seed weight) were sown in plastic pots filled with wet vermiculite to encourage the development of straight radicles. Seeds of lines with a hard seed coat were scored with a razor blade to promote water absorption. At 2 or 3 d after sowing, seed coats were removed, and several seedlings with a radicle 4 to 5 cm long were transplanted into each of two plastic trays (flooded or unflooded) for culture.

Each plastic tray measured 386 mm × 256 mm × 135 mm (Fig. 1). Doubled filter papers were folded in half (inside, No. 1; outside, No. 4A; Advantec), and seedling roots were sandwiched between a pair of folded filter papers. The filter papers were hung over horizontal strings to place their edges in the water. The flooded tray was filled with 0.1% agarose solution (Fig. 1A, C), and the unflooded tray held 2 to 3 cm of water (Fig. 1B, C). To support the roots and reduce evaporation, the filter papers were sandwiched between black plastic boards (Fig. 1C).

3. Flooding treatment under hypoxia

The flooding treatment was started 8 d after sowing. Three to five uniform seedlings per tray were selected at the cotyledon stage in each of four lines per tray were selected and the rest were removed (Fig. 1C). For the flooding treatment under hypoxia, the tray was filled with 0.1% agarose solution to just under the cotyledons (Fig. 1A, C). The solution was bubbled with N_2 gas to reduce the dissolved oxygen concentration from about 3 mg L^{-1} to about 1.2 mg L^{-1}, and to prevent convection and limit gas diffusion (Wiengweera et al., 1997). The water level in the unflooded tray was kept at 2 to 3 cm deep. To understand the hypoxic conditions, the dissolved oxygen level was measured with a DO meter (CM-51, HORIBA Ltd., Japan). All seedlings were grown in a growth chamber (220 μmol m^{-2} s^{-1}, 14 hr light/10 hr dark, 23ºC), and the experiment was replicated 2 times and we used 3 replicates per line for all lines.

4. Measurements

Seedlings were collected 7 d after treatment, and shoots were separated from roots at the cotyledonary node. The roots were scanned with the WinRHIZO software (Regent Instruments Inc.), and total root length (minus the hypocotyl), root surface area, and average root diameter were analyzed. Each fraction was dried to a constant weight at 70ºC to determine dry weight.

The rate of growth inhibition was calculated as:

[(control value – flooded value) / control value] × 100%

5. Statistical analyses

Seeds of each line (100 seeds per line) were measured for 100-seed weight (g) and the data was used for linear correlation among shoot, root, and whole-plant DW of the plants grown in unflooded condition.

We analyzed shoot DW, root DW, whole-plant DW, root DW / shoot DW, total root length, root surface area, and average root diameter by two-way analysis of variance (ANOVA) to determine the main effects and interactions between lines and treatments.

The rate of inhibition of root DW was used for linear correlation with the rates of inhibition of total root length, root surface area, and average root diameter.

Results

1. Effects of flooding on dry matter production

We examined the response of 92 soybean lines to

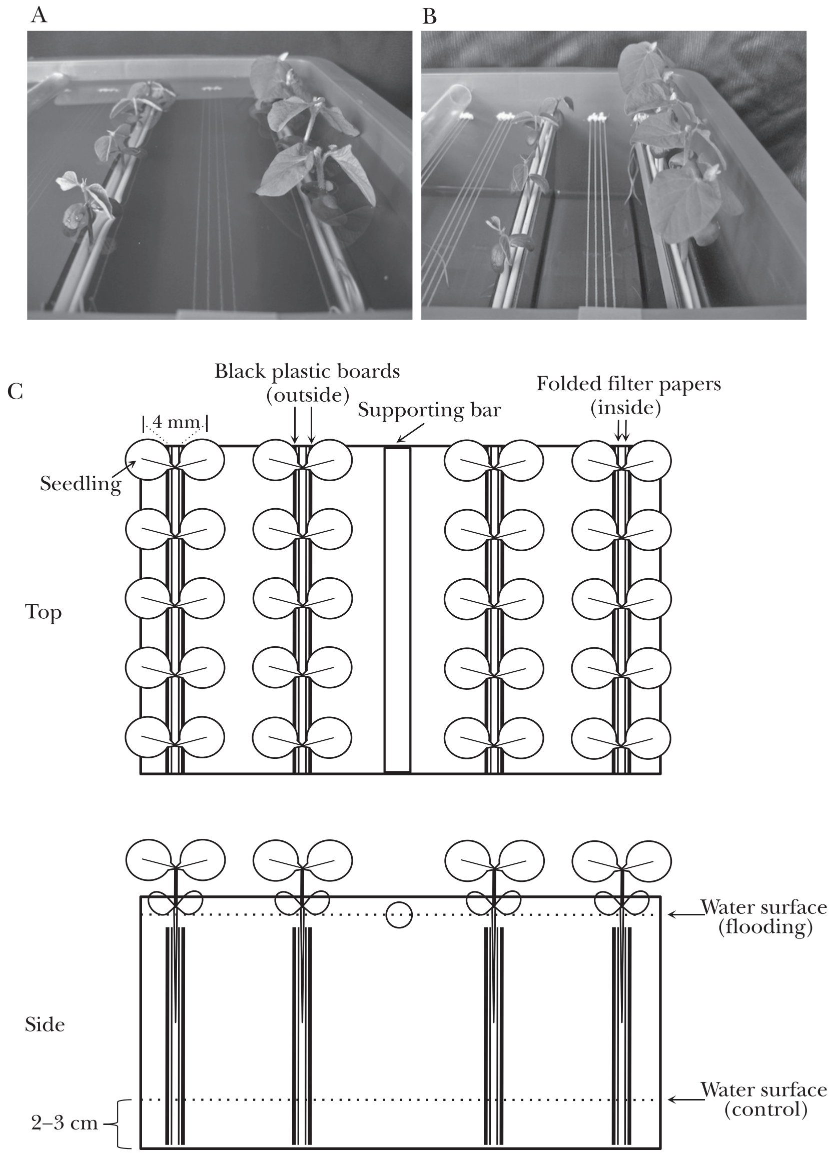

Fig. 1. The capillary watering system for soybean. Flooding was conducted at the cotyledon stage for 7 d. A, flooded (water up to hypocotyl); B, unflooded (water only at base); C, schematic views.

flooding, especially root development, by a flooding assay using a capillary watering culture system with a 0.1% agarose solution (Fig. 1). DWs of each plant part and three root traits (total root length, root surface area, and average root diameter) were determined 7 d after flooding. For hypoxic conditions, the dissolved oxygen level in the solution was 1.25 ± 0.084 mg L^{-1} at the beginning of the flooding treatment, and decreased to 0.72 ± 0.053 mg L^{-1} after the flooding treatment (by 42.4% on the average).

Most seedlings developed to the V2 stage (Fehr et al., 1971) in both flooded and control conditions. Flooding decreased the root and whole-plant DWs but not shoot DW, and means varied widely with the line in both flooded and control conditions (Tables 1, 2). The DWs in the control were significantly and positively correlated with the 100-seed weight (shoot DW, r = 0.568***; root DW, r = 0.662***; whole-plant DW, r = 0.646***; ***, P < 0.0001). The root-to-shoot DW ratio decreased under flooding (Table 2). The shoot DW inhibition was −8.5% (= promotion) on average (range, −43.5% to 12.2%). The root DW inhibition, in contrast, was positive in all lines and averaged 26.1% (0.8% to 46.2%; Table 2). Two-way ANOVA revealed that both line and treatment influenced all dry matter production (Table 3). There was a significant line × treatment interaction in root and whole-plant DW but not in shoot DW. The root-to-shoot DW ratio was significantly influenced by line, treatment, and line × treatment interaction.

Table 2. Effect of the flooding treatment on dry matter production and root traits of soybean seedlings at the cotyledon stage.

Trait	Treatment	Average	SD	Max.	Min.
Shoot DW[Z] (mg)	Flooding	100.6	34.4	185.7	10.2
	Control	93.4	33.5	181.2	9.2
	Inhibition rate (%)	−8.5	11.1	12.2	−43.5
Root DW (mg)	Flooding	67.3	19.6	100.3	8.2
	Control	94.5	32.6	156.8	8.2
	Inhibition rate (%)	26.1	10.2	46.2	0.8
Whole-plant DW[Z] (mg)	Flooding	168.0	52.3	262.8	18.4
	Control	187.8	64.7	323.0	17.5
	Inhibition rate (%)	9.0	8.8	28.9	−13.7
Root DW / Shoot DW ratio	Flooding	0.71	0.11	1.02	0.46
	Control	1.03	0.14	1.36	0.76
	Inhibition rate (%)	30.5	8.5	48.3	8.0
Total root length (cm)	Flooding	155.5	51.7	368.7	58.2
	Control	467.9	156.0	917.3	57.7
	Inhibition rate (%)	63.4	15.3	83.1	−2.7
Root surface area (cm^2)	Flooding	30.4	8.6	52.6	6.9
	Control	77.6	25.6	131.3	7.0
	Inhibition rate (%)	57.5	13.6	76.7	2.1
Root average diameter (mm)	Flooding	0.65	0.10	0.89	0.38
	Control	0.53	0.06	0.69	0.38
	Inhibition rate (%)	−23.3	13.2	4.3	−59.7

[Z]without cotyledon

2. Strong suppression of root development by flooding

Because flooding strongly inhibited root growth, we measured root traits under flooded and unflooded conditions to clarify how the flooding treatment altered soybean root development. Total root length and root surface area were severely reduced by flooding, but root diameter tended to be increased (Table 2). The inhibition of total root length and root surface area was positive in most lines, and ranged from −2.7% to 83.1% and from 2.1% to 76.7%, respectively. Two-way ANOVA revealed that line, treatment, and the line × treatment interaction strongly influenced total root length and root surface area. In contrast, average root diameter was strongly influenced by line and treatment and weakly by line × treatment interaction (Table 3).

3. Inter-line variation of the flooding effect in soybean root development

To evaluate inter-line variation of the flooding effect on root development, we analyzed the correlations of the rate of inhibition of root DW with that of total root length, root surface area, and average root diameter. Under flooding, the inhibition of root DW was linearly correlated with that of total root length and root surface area (Fig. 2A, B). G406, Iyodaizu, and Mizumoto Park No. 1, with the lowest rates of inhibition, developed long first-order lateral roots and many fine roots under flooding, which was similar to those in the control (Fig. 3), and their average root diameter did not change under flooding (Fig. 2). In contrast, Toyokomachi, Misuzudaizu, and Tachinagaha, with the highest rates of inhibition of total root length and root surface area, had shorter first-order lateral roots and less branching under flooding than in the control (Fig. 4A, B), a lower proportion of fine root length (< 0.5 mm in diameter; Fig. 4C), and a larger average root diameter under flooding (Fig. 2C).

Discussion

Short-term flooding hampered early root development in most soybean lines, although the inhibition varied widely among lines (Table 2, Fig. 2A, B). In previous studies, researchers tended to focus on aboveground parts in evaluating the tolerance or sensitivity to flooding. Our results indicate that it is important to evaluate root development as well. To dissect the effects of flooding on root morphology, we analyzed total root length, root surface area, and average root diameter. We found a linear decrease in root DW with decreasing total root length and root surface area (Fig. 2A, B). This result indicates that the suppression of root development was due mainly to the

Table 3. Results of two-way ANOVA of each trait in soybean.

Trait		F value
Root DW	line	10.21***
	treatment	1183.70***
	line × treatment	4.64***
Shoot DW[z]	line	7.69***
	treatment	37.34***
	line × treatment	0.81ns
Whole-plant DW[z]	line	8.76***
	treatment	139.84***
	line × treatment	1.65*
Root DW / shoot DW ratio	line	6.05***
	treatment	1619.28***
	line × treatment	1.96**
Total root length	line	4.88***
	treatment	2276.90***
	line × treatment	4.81***
Root surface area	line	5.83***
	treatment	2864.33***
	line × treatment	6.19***
Average root diameter	line	5.91***
	treatment	432.24***
	line × treatment	1.55*

[ns]; not significant, *; $P < 0.01$, **; $P < 0.001$, ***; $P < 0.0001$
[z]without cotyledon

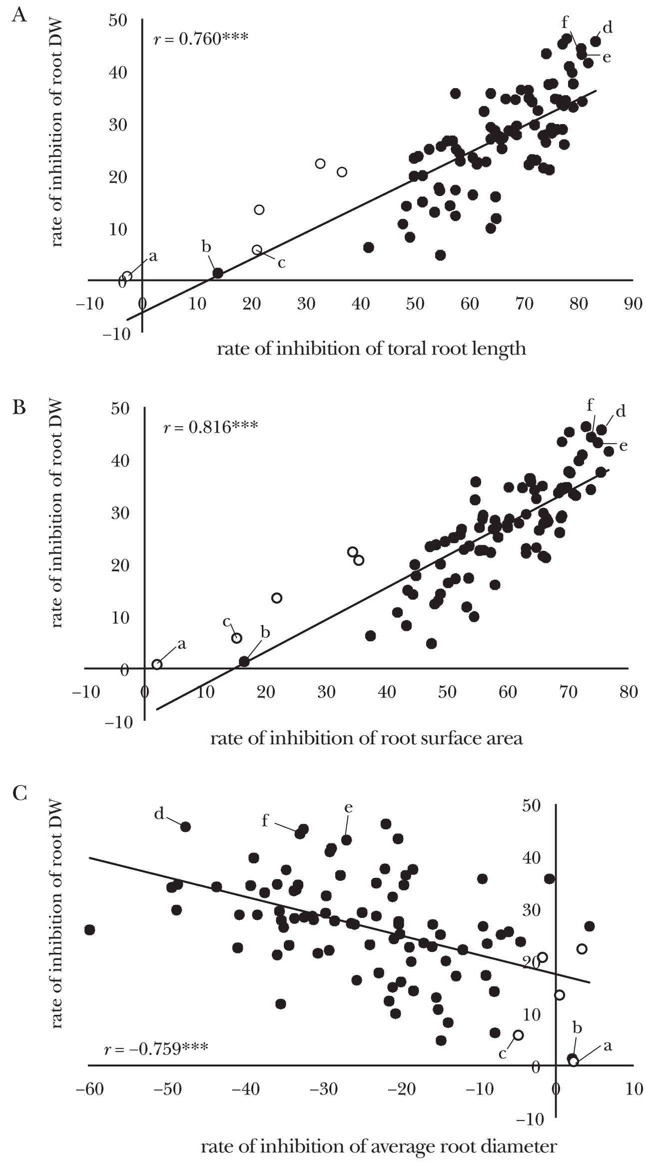

Fig. 2. Two-dimensional scatterplots of the rate of inhibition of root DW against the rates of inhibition of (A) total root length, (B) root surface area, and (C) average root diameter (***P < 0.0001). a, G406; b, Iyodaizu; c, Mizumoto Park No. 1; d, Toyokomachi; e, Tachinagaha; f, Misuzudaizu. ●, *G. max*; ○, *G. soja*.

decrease of root elongation. In fact, flood-tolerant lines continued root elongation under both flooded and control conditions (Figs. 2, 3). In contrast, flooding tended to increase average root diameter (Table 2, Fig. 2C), but flood-tolerant lines showed a similar root morphology under both flooded and control conditions (Fig. 3). Roots of flood-susceptible lines were short and thick, lacking in fine second- and higher-order lateral roots under flooding, and had a lower proportion of fine roots (< 0.5 mm in diameter) under flooding (Fig. 4). We hypothesize that flood-susceptible lines fail to develop fine roots. To prove whether flooding causes such a structural change in all soybeans we need to observe each root part. Our results show that root DW, root length, and root surface area are valuable indices of flood-tolerance of soybean plants.

The correlations of root DW with the three root traits help us to discriminate the degree of flood-tolerance in each soybean line (Fig. 2). We did not analyze the effect of flooding on shoot DW further since it was not clear. G406, Iyodaizu, and Mizumoto Park No. 1 were flood-tolerant (Fig. 2), and their root development under flooding was similar to that under control conditions (Fig. 3). G406 and Mizumoto Park No. 1 are derived from *G. soja*, which is often found in marshy areas and is regarded as flood-tolerant (Arikado, 1954). All *G. soja* lines were flood-tolerant (Fig. 2). Iyodaizu, with pale-green seeds, was probably established in Ehime Prefecture, Japan, by pure-line selection within *G. max*. The origin of Iyodaizu is unclear, and this line has never been reported as flood-tolerant. Toyokomachi, Misuzudaizu, and Tachinagaha, which produce seeds with a thin yellow seed coat, were susceptible to flooding, and showed severely suppressed root elongation under flooding(Figs. 2, 4), although Githiri et al. (2006) considered that Misuzudaizu was relatively flood-tolerant at an early vegetative growth stage in genetic analysis.

The flood tolerance of soybean has been estimated from the difference in whole-plant biomass between flooded

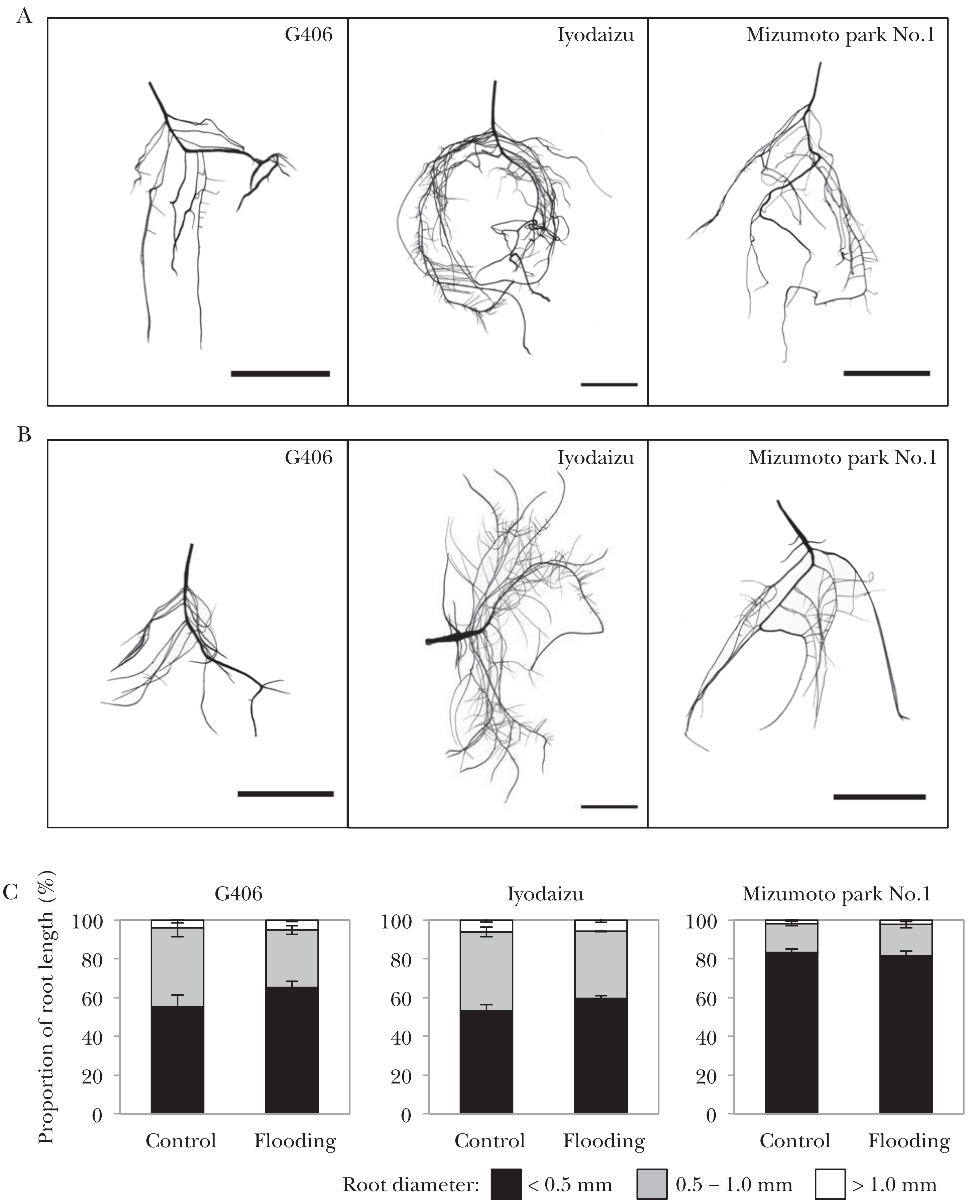

Fig. 3. Root development of flood-tolerant soybean lines after 7 d in (A) unflooded (control) and (B) flooded conditions. Bars = 5 cm. (C) Proportion of root length by diameter.

and control conditions in one or several lines (Sugimoto et al., 1988; Bacanamwo and Purcell, 1999a; Henshaw et al., 2007a, b) and the difference in pre-germination flood response between them in a large number of lines (Hou and Thseng, 1991; Wuebker et al., 2001; Sayama et al., 2009) but not the difference in root development between them in a large number of lines. This may be because the selection of flood-tolerant lines under field conditions is laborious (Mano and Omori, 2007), and the separation of roots from the soil often damages the roots. Our capillary watering culture system can overcome these problems at least partly. It offers the advantages of uniform environmental conditions, precision measurements, and screening of a large number of lines at the seedling stage.

The response of shoot growth, in contrast, was ambiguous, and the shoot DW tended to increase by flooding in some lines. Bacanamwo and Purcell (1999a) reported that the shoot biomass of soybean was similarly unaltered during the first 7 d of flooding, but decreased relative to the control by 21 d. Thus, longer flooding might affect shoot growth in our assay system. Several researchers observed aerenchyma and adventitious roots in flooded soybean plants (Bacanamwo and Purcell, 1999b; Lee et al., 2003; Shimamura et al., 2003a, b; Henshaw et al., 2007a). Both of them can help to restore the oxygen supply to the submerged parts (Bacanamwo and Purcell, 1999b; Visser and Voesenek, 2004). In the present experiment, all lines except G406 formed aerenchyma, though no or few

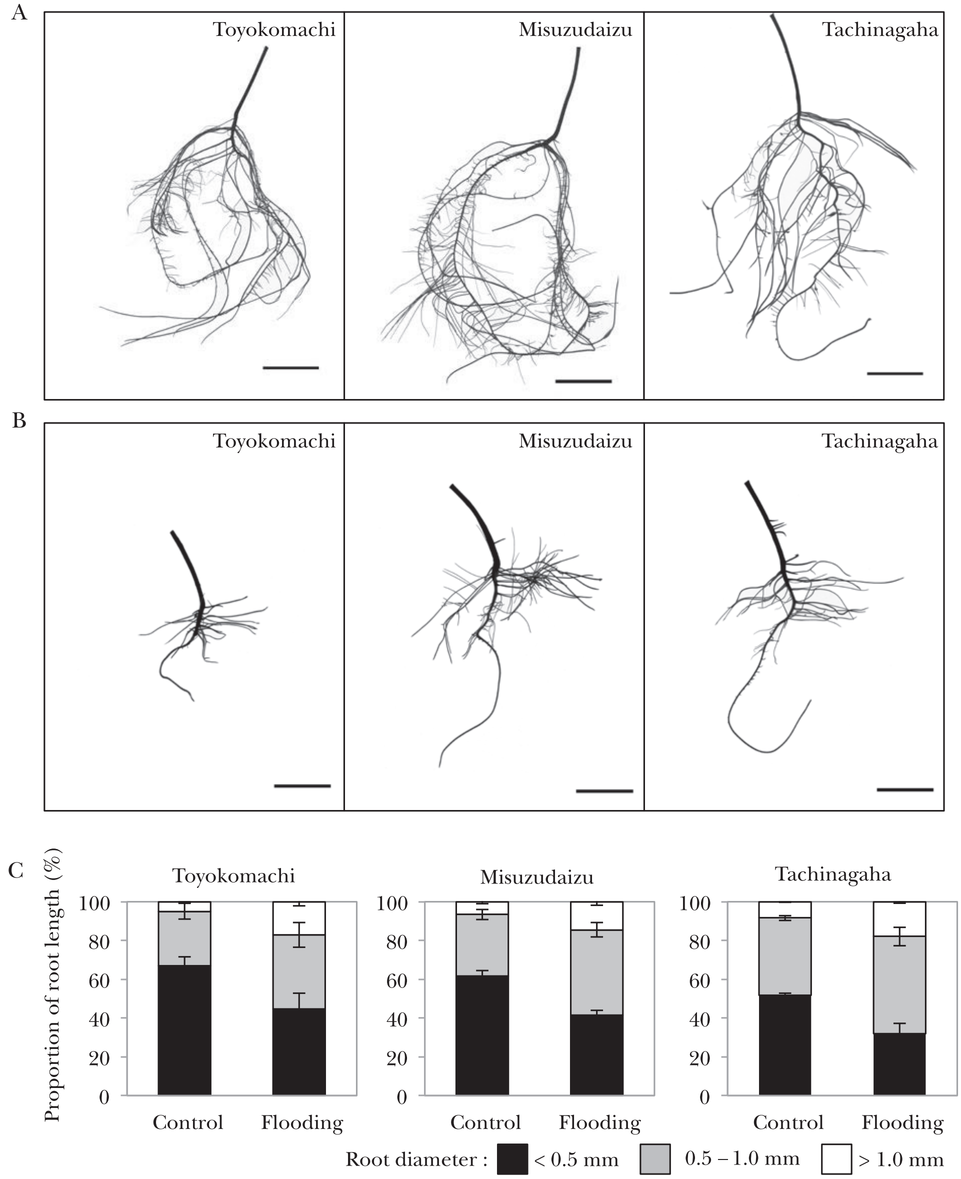

Fig. 4. Root development of flood-susceptible soybean lines after 7 d in (A) unflooded (control) and (B) flooded conditions. Bars = 5 cm. (C) Proportion of root length by diameter.

adventitious roots, in the flooded hypocotyl region (data not shown).

Domestication of plant species was achieved mainly through the observation and selection of aboveground organs, not the roots (Waines and Ehdaie, 2007). We revealed genetic variation in the effects of flooding on root development and discriminated the flood-tolerant and flood-susceptible lines. Flood-tolerant lines showed similar root growth in both flooded and control conditions, whereas flood-susceptible lines show severe inhibition of root growth under flooding. This new knowledge will be useful for understanding the effects of flooding in plants and for QTL analysis and identification of genes related to root development under flooding.

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